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Biological Agent Decontamination Technology Testing

TECHNOLOGY EVALUATION REPORT

pH-Amended Bleach
CASCAD™ Surface Decontamination Foam (Allen-Vanguard)
Decon Green
EasyDECON® 200 (EFT Holdings, Inc.)
Spor-Klenz® RTU (STERIS Corporation)
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M. WORTH CALFEE
U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
NATIONAL HOMELAND SECURITY
RESEARCH CENTER
RESEARCH TRIANGLE PARK, NC 27711

Office of Research and Development
National Homeland Security Research Center

Notice

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If you have difficulty accessing this PDF document, please contact Kathy Nickel (Nickel.Kathy@epa.gov) or Amelia McCall (McCall.Amelia@epa.gov) for assistance.

Foreword

Following the events of September 11, 2001, addressing the critical needs related to homeland security became a clear requirement with respect to EPA's mission to protect human health and the environment. Presidential Directives further emphasized EPA as the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. To support the EPA mission with respect to response and recovery from incidents of national significance, the National Homeland Security Research Center (NHSRC) was established to conduct research and deliver products that improve the capability of the Agency to carry out its homeland security responsibilities.

One specific goal of NHSRC's research is to provide information on decontamination methods and technologies that can be used in the response and recovery efforts resulting from a CBR contamination event. In recovering from an event, it is critical to identify and implement decontamination technologies that are appropriate for the given situation. In a wide-area attack scenario, the decontamination approach must be effective; while at the same time must be readily available, and easily deployed. The current study investigated several currently-available liquid and foam sporicides technologies for their ability to inactivate spores of *Bacillus anthracis* on the surface of common outdoor building materials. Information on the effectiveness of these technogies is provided to inform both decontaminant selection and implementation.

These results, coupled with additional information in separate NHSRC publications (available at www.epa.gov/nhsrc), can be used to determine whether a particular decontamination technology can be effective in a given scenario. With these factors in consideration, the best technology or combination of technologies can be chosen that meets the clean up, cost and time goals for a particular decontamination scenario.

NHSRC has made this publication available to assist the response community prepare for and recover from disasters involving chemical contamination. This research is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

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List of Acronyms and Symbols

ACQ	alkaline copper quaternary
APHA	American Public Health Association
B. anthracis	*Bacillus anthracis* (Ames strain)
BBRC	Battelle Biomedical Research Center
BSC	biosafety cabinet
C	Celsius
CFU	colony-forming unit(s)
CI	confidence interval
cm	Centimeter
DTRA	U.S. Defense Threat Reduction Agency
EPA	U.S. Environmental Protection Agency
FSP	Facility Safety Plan
g	gram
H_2O_2	hydrogen peroxide
hr	hour
L	liter
min	minute
mL	milliliter
µL	microliter
NaOCl	sodium hypochlorite
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
OCl^-	Hypochlorite ion
OPP	Office of Pesticide Programs
ORD	U.S. EPA Office of Research and Development
PBS	phosphate-buffered saline
ppm	Parts per million
psi	pounds per square inch
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolutions per minute
RTU	ready-to-use
SD	standard deviation
SDF	Surface Decontamination Foam
SE	standard error
SFW	sterile filtered water (cell-culture grade)
STS	sodium thiosulfate

TOPO	Task Order Project Officer
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program
wt	weight

Executive Summary

The U.S. Environmental Protection Agency's (EPA) National Homeland Security Research Center (NHSRC) helps to protect human health and the environment from adverse impacts of terrorist acts by carrying out performance tests on homeland security technologies. Through NHSRC's Technology Testing and Evaluation Program (TTEP), the performance of six liquid and foam decontamination technologies was evaluated for decontaminating test coupons prepared from the materials listed below. These materials include building materials typical of surfaces found outdoors in urban and residential buildings.

Nonporous materials:

- Stainless steel
- Glass
- Aluminum
- Porcelain (glazed)
- Granite (sealed surface)

Porous materials:

- Concrete
- Brick
- Asphalt paving
- Treated wood
- Butyl rubber

Experimental Procedures. Test coupons were approximately 1.9 cm by 7.5 cm in size. For testing, coupons were "contaminated" by spiking with spores of the biological agent, *Bacillus anthracis* (Ames). The technologies evaluated for their ability to inactivate *B. anthracis* (Ames) on test coupons of the listed surface materials were:

- pH-amended bleach (Ultra Clorox® Germicidal bleach diluted with commercial certified cell-culture-grade sterile filtered water (SFW) and 5% acetic acid to obtain pH-amended solution)
- Allen-Vanguard's CASCAD™ Surface Decontamination Foam (SDF)
- Decon Green
- EFT Holdings' EasyDECON® 200
- STERIS Corporation's Spor-Klenz® RTU (Ready-to-Use)
- CET, LLC's Peridox® RTU (Ready-to-Use).

With the exception of pH-amended bleach and Spor-Klenz® RTU, each decontaminant was tested using application apparatus and conditions specified by the respective vendor and according to the vendor's instructions. For pH-amended bleach, no single vendor exists. That product was tested for decontamination of outdoor surfaces using a conventional hand-pumped household garden sprayer to apply the product. For Spor-Klenz® RTU, in the absence of vendor specifications, a 500 mL hand-held plastic spray bottle was used as the applicator, consistent with other decontaminants tested. Application procedures for all the decontaminants tested are included as appendices to this report. Spray distance, humidity, and temperature were the same for all applications, and all test coupons were oriented horizontally (i.e., lying flat) for testing.

The following performance characteristics of the decontamination technologies were evaluated:

- Decontamination efficacy
    - Quantitative assessment of the decontamination efficacy for viable organisms (log reduction)
    - Qualitative assessment for residual spores on the test coupons
- Qualitative assessment of damage to material surfaces following decontamination.

Results. Results of the technology evaluation are as follows:

Table E-1 summarizes the quantitative efficacy results (as log reduction in the number of viable spores) for all six decontaminants on the 10 test materials. Efficacy results shown with the "≥" symbol indicate that complete inactivation of *B. anthracis* spores was achieved with the indicated decontaminant on that material, for all replicate test coupons. The results in Table E-1 show that the porous materials, especially concrete, asphalt paving, and treated wood, were more difficult to decontaminate than the non-porous materials. CASCAD™ SDF foam was the only one of the six decontaminants tested that achieved complete inactivation of *B. anthracis* spores on all 10 test materials. Porcelain and granite were the only two test materials on which all six decontaminants achieved complete inactivation of *B. anthracis*, although efficacy was relatively high (i.e., almost always exceeding 7 log reduction) on all of the nonporous materials.

pH-amended bleach – This liquid decontaminant was applied to the test coupons until they were fully wetted and then reapplied 15, 30, and 45 minutes after the initial application, with a total contact time before spore extraction of 60 minutes. This procedure was sufficient to maintain wetting of the stainless steel, glass, aluminum, porcelain, and butyl rubber coupons throughout the contact time. Granite, concrete, brick, asphalt, and treated wood coupons did not remain wetted throughout the entire contact time. Quantitative efficacy for *B. anthracis* was ≥ 7.62 log reduction on all five non-porous materials and ≥ 6.91 log reduction on the porous materials brick and butyl rubber. On those seven materials, inactivation of *B. anthracis* was complete, i.e., no viable spores were found on any decontaminated coupons. Efficacy on concrete, asphalt paving, and treated wood was 6.27, 3.60, and 1.90 log reduction, respectively. Only the latter two materials showed growth from decontaminated test coupons after one and seven days of incubation, consistent with the quantitative efficacy results. No visible damage was observed on any of the test materials after the 60 minute contact time with pH-amended bleach in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

CASCAD™ SDF – This decontaminant was applied to the test coupons as a foam, using a two-compartment spray bottle that mixed separate component solutions and ejected them through a fine mesh screen to create the foam. For nonporous materials, a single application and 30-minute contact time were used; for porous materials a second application was made 30 minutes after the first, and the total contact time was 60 minutes. CASCAD™ SDF foam covered both the non-porous and porous material coupons throughout the respective contact times. Quantitative efficacy of CASCAD™ SDF for *B. anthracis* was ≥ 6.80 log reduction on all ten materials. On all materials, inactivation of *B. anthracis* was complete; i.e., no viable spores were found on any decontaminated coupons. Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated test coupons of any materials. No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with CASCAD™ SDF in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

Decon Green – This liquid decontaminant was applied to all test coupons until they were fully wetted, and then reapplied 30 minutes after the initial application. The total contact time before spore extraction was 60 minutes. This application procedure was sufficient to maintain wetting of the stainless steel, glass, aluminum, porcelain, and butyl rubber coupons throughout the contact time. Granite, concrete, brick, asphalt, and treated wood coupons did not remain wetted throughout the entire contact time. The quantitative efficacy of Decon Green for *B. anthracis* was ≥ 7.32 log reduction on all five non-porous materials, and ≥ 7.25 and ≥ 6.94 log reduction on the porous materials brick and butyl rubber, respectively. No viable spores were found on any of these seven test materials after decontamination. Efficacy on concrete, asphalt, and treated wood was 4.00, 2.97, and 1.91 log reduction, respectively. Qualitative efficacy results were consistent with quantitative efficacy results, in that no viable spores were found on decontaminated coupons of seven of the ten test materials. The decontaminated coupons of concrete, asphalt, and treated wood all were positive for growth at both one and seven days' incubation. No visible damage was observed on any of the test materials after the 60 minute contact time with Decon Green in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

EasyDECON® 200 – This liquid decontaminant was applied to test coupons of glass, aluminum, and porcelain until they were fully wetted, and then reapplied 10 and 20 minutes after the initial application, with a total contact time before spore extraction of 30 minutes. This decontaminant was applied to test coupons of stainless steel and granite until they were fully wetted, and then reapplied 5, 10, 15, 20, and 25 minutes after the initial application, with a total contact time of 30 minutes. Finally, this decontaminant was applied to test coupons of the five porous materials until they were fully wetted, and then reapplied 10, 20, 30, 40, and 50 minutes after the initial application, with a total contact time of 60 minutes. These application schedules were sufficient to maintain wetting of the stainless steel, glass, aluminum, porcelain, granite, and butyl rubber coupons throughout the contact time. Concrete, brick, asphalt, and treated wood coupons did not always remain wetted throughout the entire contact time. The quantitative efficacy of EasyDECON® 200 for *B. anthracis* was ≥ 7.51 log reduction on all five non-porous materials, and approximately ≥ 7.14, ≥ 7.28 and ≥ 6.99 log reduction on the porous materials unpainted concrete, brick, and butyl rubber, respectively. No viable spores were found on any of these eight test materials after decontamination with EasyDECON® 200. Efficacy on asphalt and treated wood was 1.63 and 0.82 log reduction, respectively. Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated coupons of eight of the ten test materials. The decontaminated coupons of asphalt and treated wood all were positive for growth at both one and seven days' incubation. No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with EasyDECON® 200 in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

Spor-Klenz® RTU – This liquid decontaminant was applied to test coupons of both nonporous and porous materials until they were fully wetted, and then reapplied as necessary to keep the coupons wetted throughout the contact time. With nonporous materials, the contact time was 30 minutes and one reapplication was needed 25 minutes after the first application. With porous materials, the contact time was 60 minutes; one planned reapplication was done 30 minutes after the first application, and additional reapplications were needed 10, 25, and 50 minutes after the first application. The quantitative efficacy of Spor-Klenz® RTU for *B. anthracis* was ≥ 7.57 log reduction on the non-porous materials porcelain and granite, and ≥ 7.27 log reduction on the porous materials brick and butyl rubber. No viable spores were found on any of these four test materials after decontamination with Spor-Klenz® RTU. Efficacy was relatively high on stainless steel, glass, and aluminum (7.28, 7.36, and 7.17 log reduction, respectively), but a small number of viable spores were found on one of the replicate test coupons of each of these materials after decontamination. Efficacy on concrete, asphalt, and treated wood was 1.02, 2.56, and 6.06 log reduction, respectively. Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated coupons of porcelain, granite, brick, or butyl rubber. Decontaminated coupons of other materials were positive for growth at both one and seven days' incubation. No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with Spor-Klenz® RTU in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

Peridox® RTU – This liquid decontaminant was applied to test coupons until they were fully wetted, and then reapplied as necessary to keep the coupons wetted throughout the contact time. With nonporous materials, the contact time was 30 minutes and reapplication was needed 10 and 25 minutes after the first application. With porous materials, the contact time was 60 minutes, and reapplication was needed 10, 20, 30, 40, and 50 minutes after the first application. Quantitative efficacy of Peridox® RTU for *B. anthracis* was ≥ 6.65 log reduction on all five non-porous materials and on the porous materials treated wood and butyl rubber. No viable spores were found on any of these seven test materials after decontamination with Peridox® RTU. Efficacy was relatively high (7.22 log reduction) on asphalt paving, but a small number of viable spores were found on one of the replicate asphalt test coupons after decontamination. Efficacy of Peridox® RTU on concrete and brick was 1.39 and 3.81 log reduction, respectively. Qualitative efficacy results were largely consistent with the quantitative results, in that no growth was seen with decontaminated test coupons of the five nonporous materials and one of the porous material (treated wood). However, three test coupons of butyl rubber showed positive growth after both one and seven days' incubation. All decontaminated coupons of unpainted concrete and brick and two coupons of asphalt paving were positive for growth at both one and seven days incubation. No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with Peridox® RTU in the quantitative efficacy testing or seven days later after completion of the qualitative assessment of residual spores.

Table E-1. Summary of Quantitative Efficacy Results for *Bacillus anthracis* (Ames) by Decontaminant and Test Material

	Quantitative Efficacy (log reduction)					
Test Material	**pH-Amended Bleach**	**CASCAD™ SDF**	**Decon Green**	**Easy DECON® 200**	**Spor-Klenz® RTU**	**Peridox® RTU**
Stainless Steel	≥ 7.73	≥ 7.67	≥ 7.64	≥ 7.61	7.28	≥ 6.69
Glass	≥ 7.81	≥ 7.74	≥ 7.78	≥ 7.79	7.36	≥ 7.76
Aluminum	≥ 7.91	≥ 7.80	≥ 7.80	≥ 7.75	7.17	≥ 7.82
Porcelain	≥ 7.80	≥ 7.68	≥ 7.67	≥ 7.78	≥ 7.72	≥ 7.71
Granite	≥ 7.62	≥ 7.59	≥ 7.32	≥ 7.51	≥ 7.57	≥ 7.42
Concrete	6.27	≥ 6.93	4.00	≥ 7.14	1.02	1.39
Brick	≥ 6.91	≥ 7.40	≥ 7.25	≥ 7.28	≥ 7.27	3.81
Asphalt Paving	3.60	≥ 7.58	2.97	1.63	2.56	7.22
Treated Wood	1.90	≥ 6.97	1.91	0.82	6.06	≥ 6.99
Butyl Rubber	≥ 7.00	≥ 6.80	≥ 6.94	≥ 6.99	≥ 7.39	≥ 6.65

1.0 Introduction

NHSRC, through its Technology Testing and Evaluation Program (TTEP) works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, scientists, and permitters; and with participation of individual technology developers in carrying out performance tests on homeland security technologies. In response to the needs of stakeholders, NHSRC evaluates the performance of innovative homeland security technologies by developing test plans, conducting evaluations, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure the generation of high quality data and defensible results. NHSRC, through its TTEP, provides unbiased, third-party information supplementary to vendor-provided information that is useful to decision makers in purchasing or applying the evaluated technologies. Stakeholder involvement ensures that user needs and perspectives are incorporated into the evaluation design to produce useful performance information for each evaluated technology.

NHSRC, through its TTEP, evaluated the performance of six liquid and foam sporicidal decontamination technologies for inactivating *Bacillus anthracis* (Ames) spores on materials representative of outdoor surfaces. The technologies, which were evaluated on test coupons of ten outdoor surface materials, included the following:

- pH-amended bleach (Ultra Clorox® Germicidal bleach diluted with commercial certified cell-culture-grade sterile filtered water (SFW) and 5% acetic acid to obtain pH-amended solution)
- Allen-Vanguard's CASCAD™ Surface Decontamination Foam (SDF)
- Decon Green
- EFT Holdings' EasyDECON® 200
- STERIS Corporation's Spor-Klenz® RTU (Ready-to-Use)
- CET, LLC's Peridox® RTU (Ready-to-Use).

Testing was performed using application procedures specified by each vendor, or developed by EPA and Battelle (pH-amended bleach and Spor-Klenz® RTU) based on past experience with similar decontaminants. The application procedures for all decontaminants are included as appendices to this report. The decontaminant test procedures were specified in a peer-reviewed test/QA plan, as amended to meet the specific requirements of this evaluation. The following performance characteristics of the decontamination technologies were evaluated:

- Decontamination efficacy
  - Quantitative assessment of the decontamination efficacy for viable organisms (log reduction)
  - Qualitative assessment for residual spores on the test coupons
- Qualitative assessment of damage to material surfaces following decontamination.

2.0
Technology Description

Table 2-1 describes the decontamination technologies evaluated, based on vendor-provided information (except in the case of pH-amended bleach and Spor-Klenz® RTU) and shows the contact times used. The information provided in Table 2-1 on product composition was not confirmed in this evaluation. The application procedures used in testing of these products are included as appendices to this report.

Table 2-1. Technology Information

Product	Vendor	General Description/ Active Ingredients	Components	EPA Registration[a]	Contact Time (min)
Ultra Clorox® Germicidal Bleach	Clorox® Professional Products Co.	Sodium hypochlorite, hypochlorous acid	Sodium hypochlorite 5-6% (pH-amended by Battelle by adding acetic acid 5%)[b]	67619-8	60
CASCAD™ SDF	Allen-Vanguard	Hypochlorite	Sodium myristyl sulfate 10-30%, sodium (C14-16) olefin sulfonate 10-30%; ethanol denatured 3-9%; alcohols (C_{10}-$_{16}$) 5-10%, sodium sulfate 3-7%; sodium xylene sulfonate 1-5%; proprietary mixture of sodium and ammonia salts along with co-solvent >9%; dichloroisocyanuric acid, sodium salt 48-85%; sodium tetraborate 3-7%; sodium carbonate 10-15%.	None	30/60[c]
Decon Green	NA	Hydrogen peroxide	Three separate component solutions: A: propylene glycol, propylene carbonate, Triton® X-100 B: hydrogen peroxide, 35% C: potassium citrate monohydrate, potassium bicarbonate, potassium molybdate, propylene glycol.	1043-121	60
EasyDECON® 200	EFT Holdings, Inc.	Hydrogen peroxide	Three separate component solutions: A. benzyl C_{12}-C_{16} alkyl dimethylammonium chlorides, 5.5 to 6.5 %, N,N,N,N',N'-pentamethyl-N' tallow alkyl-trimethylenediammonium chloride, 1.5 to 2.5%; B. hydrogen peroxide, < 8.0%; C. diacetin (i.e., glycerol diacetate) 30 to 60%.	74436-1 74436-2	30/60[c]
Spor-Klenz® RTU	STERIS Corp.	Hydrogen peroxide, peracetic acid	Hydrogen peroxide 1.0%, peracetic acid 0.08%, acetic acid <10%.	1043-119	30/60[c]
Peridox® RTU	CET, LLC	Hydrogen peroxide, peracetic acid	Hydrogen peroxide 4.4%; peracetic acid 0.22 %.	81073-3	30/60[c]

[a] Registration with EPA's Office of Pesticide Programs (OPP) indicates EPA/OPP has evaluated the pesticide to show it is effective and has no unreasonable adverse effects on humans, the environment, and non-target species, and has issued a registration or license for use in the United States. Spor-Klenz® RTU and Peridox® RTU are registered as sporicides but none of the products tested is registered specifically for use against *B. anthracis*.

[b] As recommended by TTEP stakeholders, 5% acetic acid was added to the bleach to obtain a pH-amended bleach solution. Mixing 9.4 parts SFW, 1 part Ultra Clorox® Germicidal bleach, and 1 part 5% glacial acetic acid yielded a solution having a mean pH of 6.36 and mean total chlorine content of approximately 6,200 ppm. Ultra Clorox® Germicidal bleach is registered as a disinfectant but pH-amended bleach is not.

[c] Total contact times on nonporous/porous materials.

Below are brief physical descriptions of the decontamination technologies (their form, appearance as received) and preparation instructions:

- pH-Amended Bleach – Ultra Clorox® Germicidal bleach was purchased in a one-gallon container from a local retail store. The pH-adjusted decontaminant solution was prepared by mixing 9.4 parts SFW, 1 part Ultra Clorox® Germicidal bleach, and 1 part 5% glacial acetic acid. The final solution was applied using a hand-pressurized noncorroding portable garden sprayer.
- CASCAD™ SDF – This decontaminant was prepared as two separate solutions. One CASCAD™ solution was prepared by dissolving 31.2 g of GP2100 (decontaminant) in SFW and diluting to 300mL volume, and the other solution was made by dissolving 7.2 g of GPB-2100 (buffer) and 18 mL of GCE2000 (surfactant) in SFW and diluting to 300 mL volume. The application process used a vendor-supplied dual spray bottle designed to deliver equal portions of the two solutions through a single spray nozzle equipped with a diffuser mesh to produce the foam.
- Decon Green – This decontaminant is pre-packaged as three separate solutions, premeasured and ready to mix. Parts B and C were mixed together and then that mixture was added to Part A. The final solution of Decon Green has a pH of about 8 and a density of approximately 1.1 g/mL. Decon Green was applied to test coupons using a 500 mL hand-held plastic spray bottle.
- EasyDECON® 200 – This decontaminant consists of three components pre-packaged in separate containers, premeasured and ready to mix. To prepare EasyDECON® 200, the prepackaged Part One and Part Two solutions were mixed together in a clean container, and then the Part 3 solution was added and all three components were mixed thoroughly. The final solution of EasyDECON® 200 has a pH of about 9.6 to 9.9 and a density of approximately 1.08 g/mL. EasyDECON® 200 was applied to test coupons using a 500 mL hand-held plastic spray bottle.
- Spor-Klenz® RTU – Spor-Klenz® RTU is a ready-to-use clear, colorless, aqueous solution with pH 1.5 to 2.0, density of 1.01 g/mL, and a sharp acidic odor. The solution was used without dilution and applied using a 500 mL hand-held plastic spray bottle.
- Peridox® RTU – This decontaminant is an aqueous solution of 4.4 % hydrogen peroxide (H_2O_2) and 0.23% peroxyacetic acid. The product is a colorless liquid with a pH of about 2.2 and a density of approximately 1.02 g/mL. This solution was used without dilution, and was applied to test coupons using a 500 mL hand-held plastic spray bottle.

3.0 Summary of Test Procedures

Test procedures were performed in accordance with the peer-reviewed test/QA plan and are briefly summarized here.

3.1 Preparation of Test Coupons

The *Bacillus anthracis* (Ames) spores used for this testing were prepared from a qualified stock of the Ames strain at the Battelle Biomedical Research Center (BBRC). All spore lots were subject to a stringent characterization and qualification process, required by Battelle's standard operating procedure for spore production. Specifically, all spore lots were characterized prior to use by observation of colony morphology, direct microscopic observation of spore morphology and size, and determination of percent refractivity and percent encapsulation. In addition the number of viable spores was determined by colony count and expressed as colony forming units per milliliter (CFU/mL). (Theoretically, once plated onto bacterial growth media, each viable spore germinates and yields one CFU.) Variations in the expected colony phenotypes were recorded. Endotoxin concentration of each spore preparation was determined by the Limulus Ameocyte Lysate assay. Genomic DNA was extracted from the spores and DNA fingerprinting was done to confirm the genotype. The virulence of the spore lot was measured by challenging guinea pigs intradermally with a dilution series of spore suspensions, and virulence was expressed as the intradermal median lethal dose. In addition, testing was conducted for robustness of the spores via HCl resistance. The stock spore suspension was prepared in SFW at an approximate concentration of 1 x 10^9 spores/mL and stored by refrigeration at 4 °C.

B. anthracis (Ames) spores were inoculated onto test coupons in an appropriate level three biosafety cabinet (BSC) according to established BBRC procedures. Inoculated coupons were prepared prior to each day of experimental work. Coupons were placed flat in the BSC and inoculated at approximately 1 x 10^8 viable spores per coupon. This inoculation was accomplished by dispensing a 100-µL aliquot of the spore stock suspension (approximately 1 x 10^9 spores/mL) using a micropipette as 10 droplets (each of 10 µL volume) across the surface of the test coupon. This approach provided more uniform distribution of spores across the coupon surface than would be obtained through a single drop of the suspension. After inoculation, the test coupons remained undisturbed overnight in the BSC to dry. Test coupons were then decontaminated the next day, i.e., within 24 hours after inoculation.

The origin and specifications of the materials used for test coupons are shown in Table 3-1. All materials were selected as representative types of the respective building materials, based on consultation with materials suppliers. With the exception of unpainted concrete which was poured into coupons by Battelle staff and asphalt which was salvaged used material, all test

Table 3-1. Summary of Materials Used for Decontaminant Testing

Materials	Origin	Specifications
<u>**NONPOROUS**</u>		
Stainless Steel	Alro Steel Inc. Columbus, OH	Stainless, 304, 20 gauge, 2B Finish
Glass	Brooks Brothers Glass and Mirror Columbus, Ohio	C1036, 0.32 cm thick
Aluminum	Petersen Aluminum Elk Grove Village, IL	0.81 mm thick, 300 Clear Anodized
Porcelain	AF Supply Corporation New York, NY	BRIX Frimmenti DEF70 Black MOS. Tile (7 cm × 1.9 cm × 0.7 cm)
Granite	Lang Stone Co. Columbus, OH	Giallo Ornamental, Brushed finish, milled to 1.9 cm thick
<u>**POROUS**</u>		
Concrete	Wysong Concrete Fairfield, OH	5 parts sand and 2 parts cement, 1 cm thick (Battelle-made)
Brick	Hamilton Parker Co. Columbus, OH	Belcrest 560, common red, chemical resistant

Asphalt Paving	Shelly Aggregate and Asphalt Columbus, OH	Used upper layer asphalt (fine aggregate, salvaged material from urban parking lot, washed with water before cutting coupons)
Treated Wood	Lowe's Top Choice Columbus, OH	Alkaline Copper Quaternary (ACQ) treated, 5 cm x 10 cm x 2.4 m, 6.4 kg/m^3 retention (no water proofing). Item #46905, Model # TC248T225N
Butyl Rubber	Copperstate Roofing Supply Phoenix, AZ	GSSI #9897, high temperature self adhering double-sided butyl rubber sealant tape, 1.9 cm × 0.48 cm thick

coupons were made from new materials. Test coupons were approximately 1.9 x 7.5 cm in size. Coupons were sterilized before use by gamma irradiation (for asphalt, treated wood, and butyl rubber) or autoclaving (for all other materials).

Prior to testing of any decontaminants, spore recovery trials were conducted on eight of the ten test materials to define suitable spore recovery procedures. This effort was needed because of the ten materials only two (glass and concrete) had been used as test substrates in previous decontaminant tests performed by NHSRC. Spore recovery trials were conducted by inoculating three coupons of each material with *B. anthracis* (Ames) spores at the same target inoculation (1×10^8 spores/coupon (±25%)) planned for the decontaminant testing and allowing the usual overnight drying time. Those triplicate coupons, along with one blank (uninoculated) coupon, of each material were then subjected to spore recovery and enumeration using the procedures described in Section 3.2. In the spore recovery trials, the primary approach to spore recovery was agitation of a coupon in extraction solution for 15 minutes at 200 rpm. The alternative approach, which was found necessary for concrete coupons through previous testing, was sonication of the coupons in extraction solution for 45 minutes. This alternative spore recovery approach was found in the recovery trials to be necessary for granite, brick, and asphalt coupons as well.

The results of the spore recovery trials are summarized in Table 3-2, which shows the spore inoculation amount, the number of spores recovered from each of the three coupons of the eight materials, the resulting recovery values, and the average recovery (± standard deviation[SD]) on each material. The inoculation and recovered spore values in Table 3-2 are reported as CFU, as determined by the enumeration process (Section 3.2). The recovery values are the ratio of recovered to inoculated CFU, expressed as a percentage. The results in Table 3-2 arise from using the 45-minute sonication approach on granite, brick, and asphalt coupons, and the 15-minute agitation approach on coupons of the other five materials. Table 3-2 shows that average spore recovery values ranged from about 59 to 75% on the four nonporous materials (stainless steel, aluminum, porcelain, and granite) and were somewhat lower on the porous materials (brick, asphalt, treated wood, and butyl rubber), ranging from about 10 to 56%. Treated wood was the only material which exhibited an average spore recovery less than 10%. However, all recovery values are well within the target range of 1 to 150% required by the test/QA plan, and are fully sufficient for performance of decontaminant testing.

Spore recoveries were also determined for all ten coupon materials in each decontaminant test. Those recovery results are shown in the respective results chapters (Chapters 5 to 10).

3.2 Decontaminant Testing

On the day following inoculation, test coupons intended for decontamination (including blanks) were transferred into a glove box (test chamber) where the decontamination technology was applied using the apparatus and application conditions specified in the appendices of this report. The decontamination spray distance (30.5 cm), humidity (≤ 70% relative humidity), and temperature (22 ± 1°C) were the same for all applications. For most decontaminants tested, the amount of decontaminant, contact time, spray pressure, application and reapplication procedures, etc., were as specified by the vendor. For pH-amended bleach and Spor-Klenz® RTU, these parameters were chosen by EPA with input from Battelle based on previous experience and reasonable application procedures.

Five replicate test coupons (inoculated with *B. anthracis* spores and decontaminated), five replicate positive control coupons (inoculated and not decontaminated), one procedural blank (not inoculated, decontaminated), and one laboratory blank (not inoculated, not decontaminated) of each coupon material were used in testing with each decontaminant. In testing of all six decontaminants, all test coupons were oriented horizontally (i.e., lying flat). Decontaminant runoff and decontaminant pooled on top of each test coupon were captured, neutralized, and subjected to spore extraction along with the associated test coupon.

Table 3-2. Summary of Spore Recovery Trials on Eight Test Materials

Test Material	Inoculum[a] (CFU)	Recovered Spores[b] (CFU)	Recovery[c] (%)	Average Recovery (%) (± SD)
Stainless Steel	1.02×10^8	7.07×10^7 6.47×10^7 6.13×10^7	69.3 63.4 60.1	64.3 (±4.7)
Aluminum	1.02×10^8	7.77×10^7 7.77×10^7 7.57×10^7	76.2 76.2 74.2	75.5 (±1.1)
Porcelain	1.02×10^8	6.63×10^7 7.13×10^7 7.77×10^7	65.0 69.9 76.2	70.4 (±5.6)
Granite[d]	8.5×10^7	3.87×10^7 6.50×10^7 4.73×10^7	45.5 76.5 55.7	59.2 (±15.8)
Brick[d]	8.5×10^7	3.50×10^7 1.51×10^7 2.83×10^7	41.2 17.8 33.3	30.8 (±11.9)
Asphalt Paving[d]	8.5×10^7	4.89×10^7 4.87×10^7 4.43×10^7	57.5 57.3 52.1	55.7 (±3.1)
Treated Wood	8.5×10^7	8.77×10^6 8.57×10^6 7.57×10^6	10.3 10.1 8.9	9.8 (±0.8)
Butyl Rubber	1.02×10^8	2.67×10^7 3.47×10^7 3.17×10^7	26.2 34.0 31.1	30.4 (±4.0)

[a] *B. anthracis* (Ames) spores inoculated onto materials coupons, CFU = colony-forming units.
[b] Results shown for triplicate coupons of each material.
[c] Recovery is ratio of recovered to inoculated spores; see text.
[d] Spores recovered from these materials by sonication for 45 minutes; for other materials listed spore recovery was by agitation at 200 rpm for 15 minutes.

Following decontamination, each coupon (along with any associated runoff and pooled decontaminant) was transferred aseptically to a sterile 50mL conical vial containing 10 mL of sterile phosphate-buffered saline (PBS) solution with 0.1% Triton® X100 surfactant (i.e., 99.9% PBS, 0.1% Triton® X-100) and the neutralizer needed to stop the decontaminant. The required concentration of neutralizer was determined in trial runs for each decontaminant tested, according to a detailed procedure stated in the test/QA plan. In each of those trial runs, a range of neutralizer concentrations was tested to determine the concentration that most effectively stopped the action of the decontaminant (as indicated by the maximum recovery of viable spores in simulated coupon extracts). The results of those trial runs are shown in the respective results chapters (Chapters 5 to 10). As noted in Section 3.1, most coupons were then extracted by agitation on an orbital shaker for 15 minutes at approximately 200 revolutions per minute (rpm) at room temperature. For granite, concrete, brick, and asphalt the recovery of spores used an alternate procedure in which 45 minutes of sonication was used, instead of the period of agitation. For all coupons, following extraction 1 mL of the coupon extract was removed, and a series of dilutions through 10^{-7} was prepared in SFW. An aliquot (0.1 mL) of the undiluted extract and each serial dilution were then spread-plated onto tryptic soy agar plates (in triplicate) and incubated overnight at 35 to 37 ºC. Resulting colonies were enumerated within 18 to 24 hours of plating. The number of CFU/mL was determined by multiplying the average number of colonies per plate by the reciprocal of the dilution and accounting for the 0.1 mL volume of the extract or dilution that was plated.

Before further decontamination tests, the test chamber was cleaned using the vendor-supplied method for neutralizing the decontamination reagent (see the appendices to this report). If no instructions for neutralization were provided, the test chamber was cleaned following procedures established under the BBRC Facility Safety Plan.

Laboratory blanks were controlled for sterility and procedural blanks were controlled for viable spores inadvertently introduced to test coupons. The procedural blanks were spiked with an equivalent amount of 0.1 mL of "stock suspension" that did not contain the biological agent. The target acceptance criterion was that extracts of laboratory or procedural blanks were to contain no CFU. The mean percent spore recovery from each coupon material was calculated using results from positive control coupons (spiked, not decontaminated (sprayed with SFW instead of the decontaminant)) by means of the following equation:

$$\text{Mean \% Recovery} = [\text{Mean } CFU_{pc}/CFU_{spike}] \times 100 \qquad (1)$$

where Mean CFU_{pc} is the mean number of CFU recovered from five replicate positive control coupons of a single material and CFU_{spike} is the number of CFU spiked onto each of those coupons. The value of CFU_{spike} is known from enumeration of the stock spore suspension. Spore recovery was calculated for *B. anthracis* on each coupon material, and the results are included in Chapters 5 through 10.

3.3 Decontamination Efficacy

The performance or efficacy of the decontamination technology was assessed by determining the number of viable organisms remaining on each test coupon and in any decontaminant run-off from the coupon, after decontamination. Those numbers were compared to the number of viable organisms extracted from the positive control coupons, which were sprayed with SFW (the matrix for the spore suspension inoculation) instead of with the decontaminant.

The number of viable spores of *B. anthracis* in extracts of test and positive control coupons was determined to calculate efficacy of the decontaminant. Efficacy is defined as the extent (as $\log_{10}$ reduction) by which viable spores extracted from test coupons after decontamination were less numerous than the viable spores extracted from positive control coupons subjected only to an inert aqueous spray, at the same temperature and contact time as the decontaminant application. First, the logarithm of the CFU abundance from each coupon extract was determined, and then the mean of those logarithm values was determined for each set of control and associated test coupons, respectively. Efficacy of a decontaminant for a test organism on the i^{th} coupon material was calculated as the difference between those mean log values, i.e.:

$$Efficacy = \overline{(\log_{10} CFUc_{ij})} - \overline{(\log_{10} CFUt_{ij})} \qquad (2)$$

where $\log_{10} CFUc_{ij}$ refers to the j individual logarithm values obtained from the positive control coupons and $\log_{10} CFUt_{ij}$ refers to the j individual logarithm values obtained from the corresponding test coupons, and the overbar designates a mean value. In tests conducted under this plan, there were five control and five corresponding test coupons (i.e., $j = 5$) for each coupon material. In the case where no viable spores were found in any of the five test coupon extracts after decontamination, a CFU abundance of 1 was assigned, resulting in a $\log_{10}$ CFU of zero for that material. This situation occurred frequently when a decontaminant was highly effective and no viable spores were found on the decontaminated test coupons. In such cases, the final efficacy on that material was reported as greater than or equal to (≥) the value calculated by Equation 2.

The variances (i.e., the square of the standard deviation) of the $\log_{10} CFUc_{ij}$ and $\log_{10} CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 2, as follows:

$$SE = \sqrt{\frac{S^2c_{ij}}{5} + \frac{S^2t_{ij}}{5}} \qquad (3)$$

where the number 5 again represents the number j of coupons in both the control and test data sets. Thus each efficacy result is reported as a log reduction value with an associated SE value.

The significance of differences in efficacy across different coupon materials and spore types was assessed based on the 95% confidence interval of each efficacy result. The 95% confidence interval (CI) is:

$$95\%\ CI = Efficacy \pm (1.96 \times SE) \qquad (4)$$

Differences in efficacy were judged to be significant if the 95% CIs of the two efficacy results did not overlap. The efficacy results are presented in a series of tables in Chapters 5 through 10 for each decontaminant technology by coupon material.

3.4 Qualitative Assessment of Residual Spores

Based on previous decontamination studies and the spore recovery trials, it was anticipated that spores might not be completely recovered from coupons by the extraction process. Therefore, viable spores might remain on the test coupons following decontamination and extraction. As in previous decontamination studies, a qualitative assessment was performed to determine whether viable spores remained on the test coupons after extraction, including both the decontaminated test coupons and the positive control coupons not subjected to decontamination. This qualitative assessment involved different conditions and a much longer growth period than was used in the quantitative assessment of efficacy and was made to determine whether the decontaminated coupons with no growth in the quantitative measurement also showed no growth in the qualitative method.

To conduct the qualitative assessment, the test coupons from the quantitative assessment, following extraction, were transferred into tryptic soy broth culture medium and incubated for seven days at appropriate temperatures for growth. The culture media were visually inspected after one and seven days of incubation. A cloudy liquid culture after incubation indicated that viable organisms of some type remained on the coupon after decontamination and extraction. For liquid cultures in which cloudiness was observed, a loop of the liquid sample was streaked onto a tryptic soy agar plate and incubated under appropriate conditions for growth for *B. anthracis*. After incubation, the plates were examined to determine qualitatively (morphologic comparison performed visually) if the observed growth was a pure culture characteristic of the *B. anthracis* that was inoculated onto the coupons, a mixture of the inoculated organism and other endogenous organisms, or a mixture of organisms, for example molds and bacteria. Thus, the indication of the presence of viable organisms (cloudy appearance in growth medium) did not necessarily indicate the presence of residual viable organisms that had been spiked onto the test coupon.

3.5 Qualitative Assessment of Surface Damage

Trial runs were conducted before any testing with each decontaminant, using coupons that had not been spiked with spores. In these trial runs the decontaminant was applied exactly as specified in the test/QA plan and measurements were made with multiple coupons of each material type to determine the amount of the decontaminant that remained on, or ran off from, each material. This information was used in the calculation of efficacy for each respective material and in trial runs to determine the amount of neutralizing agent needed to stop the action of the decontaminant after the prescribed contact time. In addition, visual inspection of each coupon surface by two test personnel took place after the prescribed decontaminant contact time, through side-by-side comparison of the decontaminated test surface and control coupons of the same test material. Differences in color, reflectivity, and roughness were assessed qualitatively, and observations were recorded by the test personnel. The same inspection was conducted after the conclusion of the 7-day growth period that assessed qualitative efficacy (Section 3.4).

4.0 Quality Assurance/Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the test/QA plan for this evaluation, as amended, except as noted below. QA/QC procedures are summarized below.

4.1 Equipment Calibration

All equipment (e.g., pipettes, incubators, biological safety cabinets) and monitoring devices (e.g., temperature, relative humidity) used at the time of evaluation were verified as being certified, calibrated, or validated.

4.2 QC Results

Quality control efforts conducted during decontaminant testing included positive control coupons (spiked, not decontaminated), procedural blanks (not spiked, decontaminated), laboratory blanks (not spiked, not decontaminated), and spike control samples (analysis of the stock spore suspension). The results for these QC samples in each decontaminant evaluation are included in the results chapter for each respective decontaminant (i.e., see Chapters 5 through 10).

4.3 Audits

4.3.1 Performance Evaluation Audit

No performance evaluation audit was performed for *B. anthracis* (Ames) organisms because quantitative standards for these biological materials do not exist.

4.3.2 Technical Systems Audit

Battelle QA staff conducted a technical systems audit (TSA) at the BBRC on July 14 and 17, 2009, during testing of CASCAD™ SDF, to ensure that the evaluation was being conducted in accordance with the test/QA plan and the QMP.[1] As part of the TSA, test procedures were compared to those specified in the test/QA plan, and data acquisition and handling procedures were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Task Order Leader for response. The only finding of the TSA concerned the expiration date of CASCAD™ SDF solutions, as noted in Section 6.1. TSA records were permanently stored with the Battelle QA Manager.

4.3.3 Data Quality Audit

At least 10% of the data acquired during the evaluation were audited. A Battelle QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 Test/QA Plan Amendments and Deviations

The test/QA plan for this evaluation was adapted by amendment to a peer-reviewed, fully approved plan established for a previous evaluation under the TTEP program. Three amendments to the test/QA plan relevant to this testing were prepared, reviewed, approved, and distributed to all parties involved in this evaluation. Those amendments identified the materials and decontaminant technologies to be used in this evaluation and indicated the spore extraction procedures used for various materials.

Five deviations were prepared, approved, and retained in the test files for this evaluation. Two deviations were related to acceptance of spore inoculations slightly outside the target range of 1 x 10^8 spores/coupon (±25%). Those inoculations are noted in Sections 6.1 and 7.1. The third deviation was related to contamination of three laboratory blank coupons in testing of Decon Green due to a departure from usual test procedures. That occurrence is noted in Section 7.1. The fourth deviation addressed slight contamination of blank coupons by *B. anthracis* spores during testing. That occurrence is noted in Section 5.1. The fifth deviation addressed slight contamination of blank material coupons after testing, due to departure from the prescribed order of handling the coupons. That occurrence is noted in Section 10.1. None of those deviations had any significant effect on efficacy determinations for the respective decontaminants.

4.5 QA/QC Reporting

Each audit was documented, and results of the audits were submitted to the EPA (i.e., to the NHSRC Quality Assurance Manager and the Task Order Project Officer (TOPO)).

4.6 Data Review

Records and data generated in the evaluation received a QC/technical review before they were utilized in calculating or evaluating results and prior to incorporation in reports. All data were recorded by Battelle staff. The person performing the QC/technical review was involved in the experiments and added his/her initials and the date to a hard copy of the record being reviewed. This hard copy was returned to the Battelle staff member who stored the record.

5.0
pH-Amended Bleach Test Results

5.1 QC Results

In testing of pH-amended bleach, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values for *B. anthracis* spores ranged from 8.6 to 93.0%, with the lowest recovery occurring on brick.

In testing of pH-amended bleach, most procedural and laboratory blanks met the criterion of no observed CFU, with the exceptions of two laboratory blanks (not inoculated, not decontaminated) and two procedural blanks (not inoculated, decontaminated). Specifically, laboratory blank coupons for granite and brick, and procedural blank coupons for asphalt and brick, showed CFU counts of 36 to 467 CFU per coupon. This contamination likely occurred during coupon extraction, but is very slight relative to the spore inoculation of 1×10^8 spores on each test coupon. The blank CFU results do not enter into the efficacy calculations; nevertheless, a deviation was prepared regarding the acceptance of these blank coupon results. Further, decontaminated test coupons of brick and granite had no recoverable CFU following treatment, while asphalt test coupons had on average 3.83 log CFU. Thus, no contamination was apparent for brick and granite test coupons, and contamination of asphalt control coupons was on the order of 10 percent of recovered CFU from test coupons. Finally, no growth was observed in the qualitative assessment of residual spores for all procedural and laboratory blanks, which involves a much longer incubation period.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for three days of *B. anthracis* testing were 8.73×10^7/coupon, 7.73×10^7/coupon and 9.63×10^7/coupon, each within the required range.

5.2 Decontamination Efficacy

The decontamination efficacy of pH-amended bleach was evaluated for *B. anthracis* (Ames) on ten outdoor material surfaces. The following sections summarize the results found with this decontaminant.

5.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of pH-amended bleach for *B. anthracis* was ≥7.62 log reduction on all five nonporous materials, as shown in Table 5-1, and was ≥ 6.94 log reduction on the porous materials brick and butyl rubber, as shown in Table 5-2. For all of these

Table 5-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—pH-Amended Bleach on Nonporous Materials (60 minute contact time with reapplications at 15, 30, and 45 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	7.73 x 10^7	7.73 ± 0.04	69.9 ± 6.5	-
Test Coupons[c]	7.73 x 10^7	0	0	≥ 7.73 ± 0.03
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Glass				
Positive Controls	8.73 x 10^7	7.81 ± 0.06	73.9 ± 9.6	-
Test Coupons	8.73 x 10^7	0	0	≥ 7.81 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

Aluminum				
Positive Controls	8.73 x 10^7	7.91 ± 0.05	93.0 ± 10.3	-
Test Coupons	8.73 x 10^7	0	0	≥ 7.91 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Porcelain				
Positive Controls	8.73 x 10^7	7.80 ± 0.06	73.2 ± 9.6	-
Test Coupons	8.73 x 10^7	0	0	≥ 7.80 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Granite				
Positive Controls	7.73 x 10^7	7.62 ± 0.03	53.8 ± 3.9	-
Test Coupons	7.73 x 10^7	0	0	≥ 7.62 ± 0.03
Laboratory Blank	0	1.55[f]	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
[f] CFU consistent with *B. anthracis* morphology observed during spore enumeration.
"-" Not Applicable.

Table 5-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— pH-Amended Bleach on Porous Materials (60 minute contact with reapplications at 15, 30, and 45 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	8.73 x 10^7	7.47 ± 0.33	42.1 ± 28.1	-
Test Coupons[c]	8.73 x 10^7	1.20 ± 1.67	0	6.27 ± 1.49
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Brick				
Positive Controls	9.63 x 10^7	6.91 ± 0.08	8.6 ± 1.6	-
Test Coupons	9.63 x 10^7	0	0	≥ 6.91 ± 0.07
Laboratory Blank	0	1.55[f]	-	-
Procedural Blank	0	1.55[f]	-	-
Asphalt Paving				
Positive Controls	9.63 x 10^7	7.42 ± 0.26	30.8 ± 16.2	-
Test Coupons	9.63 x 10^7	3.82 ± 0.47	0.010 ± 0.009	3.60 ± 0.47
Laboratory Blank	0	0	-	-
Procedural Blank	0	2.67[f]	-	-

Treated Wood				
Positive Controls	7.73 x 10^7	7.05 ± 0.13	15.1 ± 5.0	-
Test Coupons	7.73 x 10^7	5.15 ± 0.89	0.45 ± 0.41	1.90 ± 0.79
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Butyl Rubber				
Positive Controls	7.73 x 10^7	7.00 ± 0.04	13.1 ± 1.2	-
Test Coupons	7.73 x 10^7	0	0	≥ 7.00 ± 0.03
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
[f] CFU consistent with *B. anthracis* morphology observed during spore enumeration.
"-" Not Applicable.

seven materials the efficacy result is equivalent to complete inactivation, as no viable spores were found on any decontaminated coupons. Concrete, asphalt, and treated wood exhibited lower efficacy values, at 6.27, 3.60 and 1.90 log reduction, respectively. The quantitative efficacy results are summarized in Table 5-3.

Table 5-3. Summary of Efficacy Values (Log Reduction) Obtained for pH-Amended Bleach

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	≥ 7.73
Glass	≥ 7.81
Aluminum	≥ 7.91
Porcelain	≥ 7.80
Granite	≥ 7.62
Porous	
Concrete	6.27
Brick	≥ 6.91
Asphalt Paving	3.60
Treated Wood	1.90
Butyl Rubber	≥ 7.00

5.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of the test coupons at one and seven days' post-decontamination are provided in Table 5-4. In this assessment, cultures showing positive growth (*i.e.*, a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. The qualitative results in Table 5-4 are consistent with the quantitative efficacy results shown above for pH-amended bleach on all materials. Only the asphalt and treated wood test coupons were positive for growth at both one and seven days of incubation. Although not a definitive identification, colony morphology was consistent with all observed colonies being *B. anthracis*. The laboratory and procedural blanks were all negative for growth.

5.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time with pH-amended bleach, or after seven days incubation in the qualitative efficacy test. The extraction buffer solution showed a yellowish hue when extracting the treated wood test and control coupons, most likely from wood treatment chemicals that leached out of the material.

Table 5-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores—pH-Amended Bleach

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Brick												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Treated Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
B = Blank (not inoculated with *B. anthracis* (Ames) spores); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = Coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

5.4 Other Factors

5.4.1 Operator Control

On each day of testing, pH-amended bleach was prepared according to the instructions detailed in Appendix A. To 9.4 parts water (940 mL), 1 part (100 mL) 5% acetic acid was added and mixed, then 1 part (100 mL) Ultra Clorox® Germicidal bleach was added and mixed. The pH was verified prior to use for testing as being 6.6 to 6.8. The pH-amended bleach was then transferred to a handheld garden sprayer modified with a pressure gauge to ensure that the spray was applied using 4 to 6 psi pressure. The bleach solution was then sprayed onto the test coupons and close observation of the respective material surfaces was made to ensure that they were thoroughly wetted (spray duration of approximately 3 to 5 seconds was needed to produce wetting across the surfaces of all five replicates and corresponding blank for each material type).

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1 °C). The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 70%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. Therefore, the testing chamber was always ≤ 70% RH during the decontamination of test materials with pH-amended bleach.

5.4.2 Technology Spray Deposition

pH-Amended bleach was applied according to the procedure included as Appendix A of this report. The pH-amended bleach was applied from a distance of 30.5 cm to the horizontally-oriented materials until the materials were fully wetted. Reapplication of the pH-amended bleach was made on all coupon surfaces at 15, 30, and 45 minutes after the initial application, for a total of four applications. At 60 minutes after the initial application, each material coupon was placed in the 50 mL vial that also served to collect excess decontaminant runoff. The test coupons stayed in their horizontal orientation throughout the 60 minute contact time.

To assess pH-amended bleach deposition, triplicate coupons of each test material were weighed prior to application of the pH-amended bleach in the trial runs, and these values were recorded. Then the triplicate coupons were sprayed with pH-amended bleach until fully wetted in their horizontal orientations, reapplications were made at the 15, 30, and 45 minute contact times for a total of four applications, and after 60 minutes contact time each coupon was weighed again. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the pH-amended bleach from each of the test materials is shown in Table 5-5. The total averaged value (0.75 g) over all ten materials was then used to estimate the amount of sodium thiosulfate (STS) needed to effectively neutralize the pH-amended bleach.

Table 5-5. Deposition/Runoff Weight of pH-Amended Bleach on Test Materials

Test Material	Average Deposition/Runoff Weight (g)
Nonporous	
Stainless Steel	0.63
Glass	0.64
Aluminum	0.42
Porcelain	0.51
Granite	0.33
Porous	
Concrete	0.82
Brick	1.14
Asphalt Paving	0.62
Treated Wood	1.82
Butyl Rubber	0.60
Average	*0.75*

5.4.3 Neutralization Methodology

Neutralization of the pH-amended bleach was achieved with STS. The concentrations of STS tried during the neutralization trials were 0.5, 1.0, and 1.5% in the extraction solution. These STS concentrations were based on unpublished data from previous testing. The results of the neutralization trials are shown in Table 5-6. It was determined from these trials that 0.5% STS was sufficient for neutralization of pH-amended bleach.

Table 5-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for pH-Amended Bleach

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
pH-Amended Bleach + Spores[a]	6.70×10^7	0	0
pH-Amended Bleach + PBS + Triton® X-100 + Spores[a,b]	6.70×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	6.70×10^7	7.62×10^7	100
pH-Amended Bleach + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	6.70×10^7	8.09×10^7	106.1
pH-Amended Bleach + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	6.70×10^7	7.28×10^7	95.5
pH-Amended Bleach + PBS + Triton® X-100 + 1.5% STS + Spores[ab]	6.70×10^7	8.74×10^7	114.7

[a] pH-Amended Bleach volume of 0.75 mL corresponds to mean gravimetric deposition on test materials and density of approximately 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with pH-amended bleach = 10.75 mL (10 mL PBS/Triton® X-100/STS + 0.75 mL pH-amended bleach).

6.0 CASCAD™ SDF Test Results

6.1 QC Results

In testing of CASCAD™ SDF, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. With the nonporous materials positive control recovery values ranged from 52.0 to 76.1 %, with the lowest recovery from porcelain and the highest from stainless steel. With the porous materials, positive control recovery values ranged from 6.7 to 62.5 %; the lowest recovery was from butyl rubber and the highest from asphalt paving.

In quantitative efficacy testing of CASCAD™ SDF with *B. anthracis*, all procedural and laboratory blanks met the criterion of no observed CFU. Also no growth of *B. anthracis* was observed for any procedural and laboratory blanks in the qualitative assessment of residual spores, which involves a much longer nutrient growth period. (Growth of native organisms, with colonies morphologically distinct from those of *B. anthracis*, was observed from blank coupons of two materials in the qualitative assessment as described in Section 6.2.2.)

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for three days of *B. anthracis* testing were 9.97×10^7/coupon, 9.40×10^7/coupon, and 6.17×10^7/coupon. The spike value for the third day of testing thus was outside the target range of 1×10^8 spores (± 25%). However, spore recovery values were relatively high (i.e., greater than 62%) for all materials tested on that day, allowing efficacy up to about 7.6 log reduction to be determined. As a result, no tests were repeated from that third day of testing, but a deviation was prepared noting this acceptance of a spike outside the target range.

In the TSA conducted during testing of CASCAD™ SDF, it was noted that the expiration date of the three-component solutions provided by the vendor (Allen-Vanguard) had passed. When this issue was brought to the vendor's attention, the vendor indicated that the solutions were still acceptable for use and approved proceeding with testing.

6.2 Decontamination Efficacy

The decontamination efficacy of CASCAD™ SDF was evaluated for *B. anthracis* (Ames) on ten outdoor material surfaces. The following sections summarize the results found with this decontaminant.

6.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The decontamination efficacy of CASCAD™ SDF for *B. anthracis* was greater than 6.8 log reduction on all test materials, as shown for the non-porous and porous materials in Tables 6-1 and 6-2, respectively, and summarized in Table 6-3. No viable spores were recovered from any test coupon decontaminated with CASCAD™ SDF, so all efficacy results are shown as "≥" log reduction values. The only efficacy results lower than 7.0 log reduction were on the porous materials concrete, treated wood, and butyl rubber, for which relatively low spore recoveries (i.e., < 10%) limit the efficacy result.

6.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of test, control, and blank coupons at one and seven days post-decontamination are provided in Table 6-4. In this assessment, cultures showing positive growth (*i.e.*, a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. Only *B. anthracis* colonies were found in cultures of coupons inoculated with *B. anthracis*.

Table 6-4 shows that for all ten materials, the coupons that were decontaminated with CASCAD™ SDF showed no growth for *B. anthracis*, whereas the positive control (non-decontaminated) coupons were all positive for growth. These qualitative results are consistent with the quantitative efficacy results found for CASCAD™ SDF on these materials. Laboratory and procedural blanks were all negative for growth of *B. anthracis*. The concrete laboratory blank coupons showed a cloudy growth medium at both one and seven days' incubation, but no growth of any organism was seen when the cultures were plated. The cloudiness is attributed to suspended material from the coupons (e.g., concrete dust) in the growth media. Laboratory blank coupons of brick and butyl rubber also showed cloudy growth media, but when plated the morphology of the colonies was clearly distinct from that of *B. anthracis*. As a result, laboratory blanks of concrete, brick, and butyl rubber are all shown as negative in Table 6-4.

An unusual observation was seen in the qualitative assessment with the treated wood positive control coupons. As described in Section 6.3, the extraction solutions from those coupons were yellowish, apparently due to leaching of some of the wood treatment from the coupons. The liquid culture growth assessments for those extraction solutions were negative at both Day 1 and Day 7 post-inoculation, even though the treated wood positive control coupons had not been decontaminated. On the suspicion that the wood treatment chemicals may have inhibited growth of organisms, aliquots of those negative liquid culture growth assessments were then streaked onto nutrient agar. The following day all agar plates from the liquid culture growth assessments of the treated wood positive controls clearly exhibited colonies morphologically characteristic of *B. anthracis*. This observation supports the possibility that a compound from the treated wood inhibited the growth of *B. anthracis* in liquid culture (where the concentration of the inhibitory compound was the greatest), but once a small aliquot of this liquid culture was streaked onto nutrient agar, the organism flourished. On the basis of this extra confirmation step, the treated wood positive controls were reported as positive for growth in Table 6-4. It is unclear why treated wood positive control coupon were positive for growth during testing with pH-amended bleach, and negative in these tests.

6.3 Damage to Coupons

No visible damage was observed on any of the test materials after either the 30 min or the 60 min contact time with CASCAD™ SDF. However, the extract solutions from coupons of treated wood all had a yellowish color, which is presumed to be from the wood preservative chemicals leaching out of the material. The treated wood coupons showed no visible change as a result of the extraction process. The impact of these chemicals on the qualitative assessment of residual spores is noted in Section 6.2.2, above.

6.4 Other Factors

6.4.1 Operator Control

On each day of testing, the two separate component solutions of Allen-Vanguard's CASCAD™ SDF were prepared according to the vendor's instructions as stated in Appendix B. Each half of the dual spray bottle supplied by Allen-Vanguard was then filled with one of the two component solutions, and the spray nozzle was attached to the bottle. Prior to each application, the CASCAD™ SDF spray nozzle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application, the spray nozzle was cleaned by spraying deionized water from a separate bottle. After the 30 minute contact time for nonporous materials or 60 minute contact time for the porous materials (with a reapplication at 30 minutes), each material coupon was placed in the 50 mL conical vial that also served to collect pooled CASCAD™ SDF from the coupon surface.

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1°C). The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 70%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. Therefore, the testing chamber humidity was always ≤ 70% RH in the decontamination of materials with CASCAD™ SDF.

6.4.2 Technology Spray Deposition

Allen-Vanguard's CASCAD™ SDF was applied according to the procedure included as Appendix B of this report. CASCAD™ SDF was applied from a distance of 30.5 cm to the horizontally-oriented materials until the materials were covered with an approximately 3/8-inch layer of foam. The total contact time was 30 minutes for nonporous materials and 60 minutes for porous materials. Reapplication of the CASCAD™ SDF was done only on the porous coupon surfaces at 30 minutes after the initial application, for a total of two applications.

Table 6-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—CASCAD™ SDF on Nonporous Materials (30 minute contact time, no reapplication

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	6.17×10^7	7.67 ± 0.06	76.1 ± 10.9	-
Test Coupons[c]	6.17×10^7	0	0	≥ 7.67 ± 0.05
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Glass				
Positive Controls	9.40×10^7	7.74 ± 0.10	59.7 ± 14.8	-
Test Coupons	9.40×10^7	0	0	≥ 7.74 ± 0.09
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Aluminum				
Positive Controls	9.40×10^7	7.80 ± 0.06	66.9 ± 8.9	-
Test Coupons	9.40×10^7	0	0	≥ 7.80 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Porcelain				
Positive Controls	9.40×10^7	7.68 ± 0.08	52.0 ± 9.8	-
Test Coupons	9.40×10^7	0	0	≥ 7.68 ± 0.07
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Granite				
Positive Controls	6.17×10^7	7.59 ± 0.09	64.5 ± 13.8	-
Test Coupons	6.17×10^7	0	0	≥ 7.59 ± 0.08
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 6-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— CASCAD™ SDF on Porous Materials (60 minute total contact time with reapplication at 30 min)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	9.97×10^7	6.93 ± 0.18	9.2 ± 4.4	-
Test Coupons[c]	9.97×10^7	0	0	≥ 6.93 ± 0.16
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Brick				
Positive Controls	9.97×10^7	7.40 ± 0.17	26.6 ± 9.1	-
Test Coupons	9.97×10^7	0	0	≥ 7.40 ± 0.15
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Asphalt Paving				
Positive Controls	6.17×10^7	7.58 ± 0.06	62.5 ± 9.1	-
Test Coupons	6.17×10^7	0	0	≥ 7.58 ± 0.05
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Treated Wood				
Positive Controls	9.97×10^7	6.97 ± 0.15	9.8 ± 3.2	-
Test Coupons	9.97×10^7	0	0	≥ 6.97 ± 0.13
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Butyl Rubber				
Positive Controls	9.97×10^7	6.80 ± 0.20	6.7 ± 2.2	-
Test Coupons	9.97×10^7	0	0	≥ 6.80 ± 0.18
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 6-3. Summary of Efficacy Values (Log Reduction) Obtained for CASCAD™ SDF

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	≥ 7.67
Glass	≥ 7.74
Aluminum	≥ 7.80
Porcelain	≥ 7.68
Granite	≥ 7.59
Porous	
Concrete	≥ 6.93
Brick	≥ 7.40
Asphalt Paving	≥ 7.58
Treated Wood	≥ 6.97
Butyl Rubber	≥ 6.80

To assess CASCAD™ SDF deposition, triplicate coupons of each test material were weighed prior to application of the CASCAD™ SDF, and these weights were recorded. Then the triplicate coupons were sprayed with CASCAD™ SDF until fully wetted in a horizontal orientation, and then each coupon was weighed again after its respective contact time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The resulting average deposition/runoff weight of the CASCAD™ SDF from each of the test materials is shown in Table 6-5. The average deposition amounts were 0.28 g on nonporous materials and 0.96 g on porous materials. The former quantity was then used in trials to estimate the amount of STS needed to effectively neutralize the CASCAD™ SDF in testing with nonporous materials. However, an amount of 1.29 g was erroneously used in the corresponding neutralization trial for porous materials. The results of the neutralization trials, including the use of that erroneous CASCAD™ SDF amount to represent deposition on porous materials, are presented in the next section.

Table 6-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores—CASCAD™ SDF

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Concrete												
Positive Controls	+	+	+	+	+	-[c]	+	+	+	+	+	-[c]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Brick												
Positive Controls	+	+	+	+	+	-[d]	+	+	+	+	+	-[d]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Treated Wood												
Positive Controls[e]	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-[d]	+	+	+	+	+	-[d]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
B = Blank (not inoculated with *B. anthracis* (Ames) spores); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = Coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.
[c] A cloudy extraction solution was observed, but no organisms were detected when solution was plated (see text).
[d] Positive growth was indicated by a cloudy solution after incubation, but morphology of organisms not consistent with *B. anthracis* (see text).
[e] Positive control coupons of treated wood showed no growth in one-day or seven-day incubation, but showed growth consistent with *B. anthracis* morphology when culture was plated (see text).

Table 6-5. Deposition/Runoff Weights of CASCAD™ SDF on Test Materials

Test Material	Average Deposition/ Runoff Weight (g)
Nonporous	
Stainless Steel	0.11
Glass	0.23
Aluminum	0.23
Porcelain	0.56
Granite	0.28
Average	*0.28*
Porous	
Concrete	0.75
Brick	1.40
Asphalt Paving	0.60
Treated Wood	1.59
Butyl Rubber	0.46
Average	*0.96*

6.4.3 Neutralization Methodology

Neutralization of CASCAD™ SDF was achieved with STS. For both nonporous and porous materials, the concentrations of STS tested in the neutralization trials were 0.5, 1.0, and 1.5% in the PBS/Triton® X-100 extraction solution. This range of STS concentrations was chosen based on previous experience with CASCAD™ SDF. The results of the neutralization trials are shown in Tables 6-6 and 6-7, for the nonporous and porous materials, respectively. On the basis of these results 0.5% STS was chosen for neutralization of CASCAD™ SDF in testing with the nonporous materials and 1.0% STS was chosen for testing with the porous materials. The use of an erroneously large amount of CASCAD™ SDF to represent deposition on porous materials (i.e., 1.29 g rather than 0.96 g; see Section 6.4.2) does not affect the results in Table 6-7. The 1% STS concentration chosen was sufficient to neutralize 1.29 g of CASCAD™ SDF, and therefore would certainly neutralize the average actual deposition of 0.96 g of CASCAD™ SDF on the porous coupons. On the basis of the results in Table 6-7, the 1% STS concentration was used for neutralization in the tests with porous materials.

Table 6-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for CASCAD™ SDF on Nonporous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
CASCAD™ SDF + Spores[a]	9.47×10^7	0	0
CASCAD™ SDF + PBS + Triton® X-100 + Spores[a,b]	9.47×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	9.47×10^7	1.04×10^8	100
CASCAD™ SDF + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	9.47×10^7	1.06×10^8	102.1
CASCAD™ SDF + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	9.47×10^7	9.24×10^7	89.1
CASCAD™ SDF + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	9.47×10^7	9.46×10^7	91.2

[a] CASCAD™ SDF volume of 0.28 mL corresponds to mean gravimetric deposition on nonporous materials and liquid density of approximately 1.0 g/mL.

[b] 10 mL volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 10.28 mL (10 mL PBS/Triton® X-100/STS + 0.28 mL CASCAD™ SDF).

Table 6-7. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for CASCAD™ SDF on Porous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
CASCAD™ SDF + Spores[a]	8.67×10^7	0	0
CASCAD™ SDF + PBS + Triton® X-100 + Spores[a,b]	8.67×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	8.67×10^7	1.06×10^8	100
CASCAD™ SDF + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	8.67×10^7	8.47×10^7	79.6
CASCAD™ SDF + PBS + Triton®X-100 + 1.0% STS + Spores[a,b]	8.67×10^7	9.76×10^7	91.7
CASCAD™ SDF + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	8.67×10^7	9.70×10^7	91.1

[a] CASCAD™ SDF volume of 1.29 mL corresponds to mean gravimetric deposition on porous materials and liquid density of approximately 1.0 g/mL (see text).

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with CASCAD™ SDF = 11.29 mL (10 mL PBS/Triton® X-100/STS + 1.29 mL CASCAD™ SDF).

7.0 Decon Green Test Results

7.1 QC Results

In testing of Decon Green, all positive control results were within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values on nonporous materials ranged from about 27 to 90%, with the lowest recovery on granite and the highest on aluminum; positive control recovery values on porous materials ranged from about 7 to 57%, with the lowest recovery on concrete and the highest on asphalt.

Laboratory blanks showed no indication of *B. anthracis*, except for the laboratory blanks of glass, aluminum, and porcelain from one of the Decon Green test days, which were found to be contaminated with small numbers of *B. anthracis* spores. This contamination apparently occurred because these blanks (*i.e*., non-inoculated material coupons) were placed with the inoculated test and positive control coupons during the overnight drying period. In previous testing, the blank coupons were segregated from the inoculated coupons during drying and only placed into the test chamber after the inoculated coupons had been placed there. That approach avoided transfer of any spores to blank coupons by agitation or air movement during drying, or by contact with the inoculated coupons during the transfer to the chamber for decontaminant testing. However, this procedure was not followed for the blank coupons on the day of Decon Green testing that involved these three materials. Consequently, those blanks showed positive growth in the qualitative assessment of residual spores. A report detailing the deviation from the test/QA plan was prepared noting this departure from procedures. No viable spores were detected in the quantitative efficacy testing, and no growth was observed in the qualitative assessment of residual spores, for any of the procedural blanks of any materials (i.e., not inoculated with spores but subjected to Decon Green application).

Spike control samples were taken from the spore suspension on each day of testing, serially diluted, nutrient plated, and counted to establish the spore density used to inoculate the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to an inoculation of 1×10^8 spores (± 25%) on each test coupon. The actual inoculation values for three days of *B. anthracis* testing were 7.13×10^7/coupon, 7.13×10^7/coupon and 8.30×10^7/coupon. It is unknown why the spore suspension density was below the target criterion for the first two testing days. Spore recovery results were good on those days, allowing determination of quantitative efficacy of 6.67 log reduction or more. Consequently, the tests from those days were not repeated, but a deviation from the test/QA plan was prepared noting this acceptance of a spike outside the target range.

7.2 Decontamination Efficacy

The decontamination efficacy of Decon Green was evaluated for *B. anthracis* (Ames) on ten outdoor material surfaces. The following sections summarize the results found with this decontaminant.

7.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The results for decontamination efficacy of Decon Green on nonporous and porous materials are shown in Tables 7-1 and 7-2, respectively, and summarized in Table 7-3. Decontamination efficacy was ≥ 7.3 log reduction for all five of the nonporous materials, as shown in Table 7-1. No viable spores were found on any of the nonporous materials after decontamination with Decon Green. The decontamination efficacy of Decon Green was not as consistent for the porous materials, as is shown in Table 7-2. Efficacy was ≥ 7.3 and ≥ 6.9 log reduction, respectively, on brick and butyl rubber, whereas concrete, asphalt, and treated wood had log reductions of 4.0, 3.0, and 1.9, respectively. Table 7-3 lists all efficacy results from both nonporous and porous materials.

7.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupon extracts at one and seven days post-decontamination are provided in Table 7-4 for coupons inoculated with *B. anthracis* (Ames). In this assessment, cultures showing positive growth (*i.e*., a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. Only colonies indicative of *B. anthracis* were observed in cultures of coupons inoculated with *B. anthracis*.

Table 7-4 shows qualitative efficacy results for all materials which are consistent with the quantitative efficacy results reported in Section 7.2.1 (Table 7-3). For all five nonporous materials, and for brick and butyl rubber, no growth was observed from the decontaminated test coupons after either one or seven days' incubation. The decontaminated coupons of concrete, asphalt, and treated wood all were positive for growth at both one and seven days' incubation. One of the concrete test replicates (S5) was negative for growth after one day of incubation, but was positive for growth and verified as *B. anthracis* after seven days as shown in Table 7-4.

Table 7-4 also shows that the laboratory and procedural blanks were all negative for growth with the exception of the laboratory blanks of glass, porcelain, and aluminum. Those blank coupons were inadvertently left with the inoculated coupons during the overnight drying period, as described in Section 7.1, and apparently became contaminated.

Table 7-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—Decon Green on Nonporous Materials (60 minute contact time with reapplication at 30 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	8.30×10^7	7.64 ± 0.04	52.9 ± 4.2	-
Test Coupons[c]	8.30×10^7	0	0	≥7.64 ± 0.03
Laboratory Blank[d]	0	-	-	-
Procedural Blank[e]	0	-	-	-
Glass				
Positive Controls	7.13×10^7	7.78 ± 0.04	84.6 ± 6.9	-
Test Coupons	7.13×10^7	0	0	≥7.78 ± 0.03
Laboratory Blank	0	3.47[f]	-	-
Procedural Blank	0	0	-	-
Aluminum				
Positive Controls	7.13×10^7	7.80 ± 0.07	90.0 ± 13.6	-
Test Coupons	7.13×10^7	0	0	≥7.80 ± 0.06
Laboratory Blank	0	3.49[f]	-	-
Procedural Blank	0	0	-	-
Porcelain				
Positive Controls	7.13×10^7	7.67 ± 0.09	66.2 ± 14.4	-
Test Coupons	7.13×10^7	0	0	≥7.67 ± 0.08
Laboratory Blank	0	3.63[f]	-	-
Procedural Blank	0	0	-	-
Granite				
Positive Controls	8.30×10^7	7.32 ± 0.19	27.2 ± 12.3	-
Test Coupons	8.30×10^7	0	0	≥7.32 ± 0.17
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
[f] Contamination of blanks with *B. anthracis* occurred during drying (see text).
"-" Not Applicable.

Table 7-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— Decon Green on Porous Materials (60 minute contact time with reapplication at 30 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	7.13×10^7	6.67 ± 0.13	6.9 ± 2.1	-
Test Coupons[c]	7.13×10^7	2.67 ± 1.90	0.020 ± 0.032	4.00 ± 1.67
Laboratory Blank[d]	0	0	-	-
Procedural Blank[e]	0	0	-	-
Brick				
Positive Controls	7.13×10^7	7.25 ± 0.04	24.7 ± 2.1	-
Test Coupons	7.13×10^7	0	0	≥7.25 ± 0.03
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Asphalt Paving				
Positive Controls	8.30×10^7	7.67 ± 0.06	57.0 ± 7.9	-
Test Coupons	8.30×10^7	4.71 ± 0.23	0.069 ± 0.040	2.97 ± 0.21
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Treated Wood				
Positive Controls	7.13×10^7	6.93 ± 0.23	13.1 ± 5.5	-
Test Coupons	7.13×10^7	5.02 ± 1.69	0.77 ± 0.69	1.91 ± 1.50
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-
Butyl Rubber				
Positive Controls	7.13×10^7	6.94 ± 0.05	12.3 ± 1.4	-
Test Coupons	7.13×10^7	0	0	≥6.94 ± 0.04
Laboratory Blank	0	0	-	-
Procedural Blank	0	0	-	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 7-3. Summary of Efficacy Values (Log Reduction) Obtained for Decon Green

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	≥7.64
Glass	≥7.78
Aluminum	≥7.80
Porcelain	≥7.67
Granite	≥7.32
Porous	
Concrete	4.00
Brick	≥7.25
Asphalt Paving	2.97
Treated Wood	1.91
Butyl Rubber	≥6.94

The same observation noted in testing of CASCAD™ SDF (Section 6.2.2) was seen in the qualitative assessment with Decon Green, with both the test and positive control coupons of treated wood. The liquid culture growth assessments for treated wood test and positive control coupons were negative after both one and seven days' incubation, even though the positive control coupons had not been decontaminated and the test coupons had been minimally decontaminated (i.e., Decon Green efficacy on treated wood was only 1.91 log reduction). As noted in Section 7.3, the growth assessment solutions from the treated wood coupons had a slight yellow hue. To investigate the possibility of inhibition from the wood treatment itself, these visibly negative liquid culture growth assessments from the Decon Green testing were plated on nutrient agar. By the following day all agar plates clearly showed colonies exhibiting *B. anthracis* morphology. This observation strongly suggested that an inhibitory compound from the treated wood prevented the growth of *B. anthracis* in liquid culture (where the concentration of the inhibitory compound was the greatest), but the organism flourished once a small amount of the liquid culture was plated out onto nutrient agar. Therefore, the positive control and test coupons of treated wood were indicated as positive for growth in Table 7-4 because the plating step established the presence and viability of *B. anthracis* in the liquid culture.

7.3 Damage to Coupons

No visible damage was observed on the test materials after the 60 min contact time with Decon Green, or after seven days' incubation in the qualitative efficacy test. The extract solutions of treated wood coupons had a noticeable yellowish hue, probably due to leaching of treatment chemicals from the coupon material.

7.4 Other Factors

7.4.1 Operator Control

On each day of testing, the three components of Decon Green were prepared according to the vendor's explicit instructions, as stated in Appendix C. Prior to each application, the Decon Green spray nozzle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application, the spray nozzle was removed from the bottle and any residual Decon Green was removed by repeated pulls on the trigger of the spray nozzle. The spray nozzle was then placed into a reservoir that contained only sterile, cell culture-grade water so as to completely clean out the spray nozzle until its next use. All material coupons were oriented horizontally (i.e., lying flat) and stayed in that orientation throughout the entire contact time.

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1 °C). The relative humidity (RH) inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 70%, the dehumidification system attached to the test chamber was actuated until the RH dropped below 70%. Therefore, the test chamber RH was always ≤ 70% RH during the decontamination of test materials with Decon Green.

7.4.2 Technology Spray Deposition

Decon Green was applied according to the procedure included as Appendix C of this report. Decon Green was applied from a distance of 30.5 cm to the horizontally-oriented materials until the materials were fully wetted. Reapplication of the Decon Green was made on both the nonporous and porous coupon surfaces at 30 minutes after the first application, for a total of two applications. After the 60 minute contact time, each material coupon was carefully placed into the respective extraction tube that also served to collect excess decontaminant runoff.

To assess Decon Green deposition, triplicate coupons of each test material were weighed prior to application of the Decon Green in the trial runs, and these values were recorded. Then the triplicate coupons were sprayed with Decon Green until fully wetted in a horizontal orientation, sprayed again 30 minutes later, and then each coupon was weighed again after the 60 minute contact time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the Decon Green from each of the test materials is shown in Table 7-5. The average deposited amounts of 0.65 g on nonporous materials and 0.75 g on porous materials were then used in trials to determine the amount of sodium thiosulfate (STS) needed to effectively neutralize the Decon Green.

Table 7-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores —Decon Green

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	+[c]	+	+	+	+	+	+[c]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	+[c]	+	+	+	+	+	+[c]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	+[c]	+	+	+	+	+	+[c]
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	-	-	+	+	+	+	+	-
Brick												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Treated Wood												
Positive Controls[d]	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons[d]	+	+	+	+	+	-	+	+	+	+	+	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5. B = Blank (not inoculated with *B. anthracis* (Ames)); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = Coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.
[c] These laboratory blanks inadvertently exposed to inoculated coupons during drying (see text).
[d] Treated wood coupons showed no growth in one-day or seven-day incubation, but showed growth consistent with *B. anthracis* morphology when culture was plated (see text).

7.4.3 Neutralization Methodology

Neutralization of Decon Green was achieved with STS. The concentrations of STS used during the neutralization trials were 2.0, 2.5, and 3.0% in the PBS/Triton X-100 extraction solution, because initial trials showed that lower concentrations were inadequate to neutralize the Decon Green. The results of the neutralization trials are shown for nonporous test materials in Table 7-6 and for the porous test materials in Table 7-7. From these results it was concluded that 2.0% STS and 3.0% STS concentrations in the extraction solution were sufficient for neutralization of Decon Green for the nonporous and porous materials, respectively.

Table 7-5. Deposition/Runoff Weight of Decon Green on Test Materials

Test Material	Average Deposition/ Runoff Weight (g)
Nonporous	
Stainless Steel	0.52
Glass	0.81
Aluminum	0.62
Porcelain	0.92
Granite	0.37
Average	*0.65*
Porous	
Concrete	0.39
Brick	0.79
Asphalt Paving	0.51
Treated Wood	1.05
Butyl Rubber	1.03
Average	*0.75*

Table 7-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Decon Green on Nonporous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Decon Green + Spores[a]	8.70×10^7	0	0
Decon Green + PBS + Triton® X-100 + Spores[a,b]	8.70×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	8.70×10^7	8.10×10^7	100
Decon Green + PBS + Triton® X-100 + 2.0% STS + Spores[a,b]	8.70×10^7	7.68×10^7	94.8
Decon Green + PBS + Triton® X-100 + 2.5% STS + Spores[a,b]	8.70×10^7	7.12×10^7	87.9
Decon Green + PBS + Triton® X-100 + 3.0% STS + Spores[a,b]	8.70×10^7	7.42×10^7	91.6

[a] Decon Green volume of 0.65 mL corresponds to mean gravimetric deposition on nonporous materials, and density of approximately 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Decon Green = 10.65 mL (10 mL PBS/Triton® X-100/STS + 0.65 mL Decon Green).

Table 7-7. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Decon Green on Porous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Decon Green + Spores[a]	8.70×10^7	0	0
Decon Green + PBS + Triton® X-100 + Spores[a,b]	8.70×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	8.70×10^7	7.89×10^7	100
Decon Green + PBS + Triton® X-100 + 2.0% STS + Spores[ab]	8.70×10^7	7.16×10^7	90.8
Decon Green + PBS + Triton® X-100 + 2.5% STS + Spores[a,b]	8.70×10^7	7.70×10^7	97.6
Decon Green + PBS + Triton® X-100 + 3.0% STS + Spores[a,b]	8.70×10^7	8.22×10^7	104.1

[a] Decon Green volume of 0.75 mL corresponds to mean gravimetric deposition on porous materials, and density of approximately 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Decon Green = 10.75 mL (10 mL PBS/Triton® X-100/STS + 0.75 mL Decon Green).

8.0 EasyDECON® 200 Test Results

8.1 QC Results

In testing of EasyDECON® 200, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values on the nonporous materials ranged from 40 to 74%, with the lowest recovery occurring on granite and the highest on glass. Positive control recovery values on the porous materials ranged from 12 to 39%, with the lowest recovery occurring on butyl rubber and the highest on asphalt paving.

In testing of EasyDECON® 200, all procedural and laboratory blanks met the criterion of no observed CFU in quantitative efficacy testing with *B. anthracis*. No growth was observed in the qualitative assessment of residual spores for all procedural and laboratory blanks.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for three days of *B. anthracis* testing were all within that criterion, at 8.40×10^7/coupon, 8.07×10^7/coupon and 8.77×10^7/coupon, respectively.

8.2 Decontamination Efficacy

The decontamination efficacy of EasyDECON® 200 was evaluated for *B. anthracis* (Ames) on ten outdoor material surfaces. The following sections summarize the results found with this decontaminant.

8.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The results for decontamination efficacy of EasyDECON® 200 on nonporous and porous materials are shown in Tables 8-1 and 8-2, respectively, and summarized in Table 8-3. A relatively large deposition amount (approximately 2 g per coupon) was recommended by the vendor of EasyDECON® 200, but it was difficult to achieve such a high deposition rate in practice. Testing of EasyDECON® 200 began with nonporous materials, using a procedure that called for three applications of the decontaminant. The resulting deposited amount of EasyDECON® 200 was lower than

Table 8-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—EasyDECON® 200 on Nonporous Materials (30 minute contact time with reapplication at 10 and 20 minutes; or 30 minute contact time with reapplication at 5, 10, 15, 20, and 25 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	8.07×10^7	7.61 ± 0.03	50.4 ± 3.9	-
Test Coupons[c]	8.07×10^7	0	0	≥ 7.61 ± 0.03
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Glass[f]				
Positive Controls	8.40×10^7	7.79 ± 0.05	74.2 ± 8.4	-
Test Coupons	8.40×10^7	0	0	≥ 7.79 ± 0.04
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Aluminum[f]				
Positive Controls	8.40×10^7	7.75 ± 0.07	67.2 ± 11.7	-
Test Coupons	8.40×10^7	0	0	≥ 7.75 ± 0.06
Laboratory Blank	0	0	0	-

Procedural Blank	0	0	0	-
Porcelain[f]				
Positive Controls	8.40 x 10^7	7.78 ± 0.01	71.3 ± 2.0	-
Test Coupons	8.40 x 10^7	0	0	≥ 7.78 ± 0.01
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Granite				
Positive Controls	8.07 x 10^7	7.51 ± 0.05	40.0 ± 5.0	-
Test Coupons	8.07 x 10^7	0	0	≥ 7.51 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
[f] This material tested with three applications of EasyDECON 200®; others tested with six applications.
"-" Not Applicable.

Table 8-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— EasyDECON 200 on Porous Materials (60 minute contact time with reapplications at 10, 20, 30, 40, and 50 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	8.77 x 10^7	7.14 ± 0.03	15.6 ± 1.2	-
Test Coupons[c]	8.77 x 10^7	0	0	≥ 7.14 ± 0.03
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Brick				
Positive Controls	8.77 x 10^7	7.28 ± 0.23	24.6 ± 13.8	-
Test Coupons	8.77 x 10^7	0	0	≥ 7.28 ± 0.20
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Asphalt Paving				
Positive Controls	8.07 x 10^7	7.47 ± 0.16	38.5 ± 11.5	-
Test Coupons	8.07 x 10^7	5.84 ± 0.05	0.87 ± 0.096	1.63 ± 0.14
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Treated Wood				
Positive Controls	8.77 x 10^7	6.96 ± 0.30	12.9 ± 11.3	-

Test Coupons	8.77 x 10^7	6.13 ± 0.04	1.55 ± 0.16	0.82 ± 0.26
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Butyl Rubber				
Positive Controls	8.77 x 10^7	6.99 ± 0.12	11.6 ± 3.3	-
Test Coupons	8.77 x 10^7	0	0	≥ 6.99 ± 0.10
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[b] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 8-3. Summary of Efficacy Values (Log Reduction) Obtained for EasyDECON® 200

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	≥ 7.61
Glass[a]	≥ 7.79
Aluminum[a]	≥ 7.75
Porcelain[a]	≥ 7.78
Granite	≥ 7.51
Porous	
Concrete	≥ 7.14
Brick	≥ 7.28
Asphalt Paving	1.63
Treated Wood	0.82
Butyl Rubber	≥ 6.99

[a] These three materials tested with three applications of EasyDECON® 200; all others tested with six applications.

expected, but nevertheless for three nonporous materials (glass, aluminum, and porcelain) complete kill of *B. anthracis* spores was achieved. To increase the deposition rate, a revised procedure of six applications was then developed and used with the vendor's approval on all the porous materials and on the two other nonporous materials (stainless steel and granite). Both application procedures are described in Appendix D, and footnotes to Tables 8-1 and 8-3 indicate which materials were tested with three and which with six applications of EasyDECON® 200.

The decontamination efficacy of EasyDECON® 200 for *B. anthracis* was ≥ 7.51 log reduction with all of the nonporous materials, as shown in Table 8-3. No viable spores were found on any of the nonporous materials after decontamination with EasyDECON® 200. However, the decontamination efficacy of EasyDECON® 200 was not as consistent with the porous materials, as shown in Table 8-3. Efficacy values of about 7.0 log reduction were achieved with unpainted concrete, brick, and butyl rubber, and no viable spores were found on these materials after decontamination with EasyDECON® 200. In contrast, efficacy values of 1.63 and 0.82 log reduction were found with asphalt paving and treated wood, respectively.

8.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Table 8-4 for coupons spiked with *B. anthracis* (Ames). In this assessment, cultures showing positive growth (*i.e*., a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. Only colonies consistent with the morphology of *B. anthracis* were found in cultures of coupons inoculated with *B. anthracis*.

Table 8-4 shows qualitative efficacy results for all materials which are consistent with the quantitative efficacy results reported in Section 8.2.1 (Table 8-3). For all five nonporous materials, and for concrete, brick, and butyl rubber, no growth was observed from the decontaminated test coupons after either one or seven days' incubation. The decontaminated coupons of asphalt and treated wood all were positive for growth at both one and seven days' incubation, consistent with the relatively low quantitative efficacy results for those materials (Table 8-3). Table 8-4 also shows that all laboratory and procedural blanks were negative for growth.

The same observation noted in testing of CASCAD™ SDF (Section 6.2.2) and Decon Green (Section 7.2.2) was seen in the qualitative assessment with EasyDECON® 200, with both the test and positive control coupons of treated wood. That is, the liquid culture growth assessments for treated wood test and positive control coupons were negative after both one and seven days' incubation, even though the positive control coupons had not been decontaminated and the test coupons had been minimally decontaminated (i.e., EasyDECON® 200 efficacy on treated wood was only 0.82 log reduction). As noted in Section 8.3, the growth assessment solutions from the treated wood coupons had a slight yellow hue. These visibly negative liquid culture growth assessments from the EasyDECON® 200 testing were plated on nutrient agar, and by the following day all agar plates clearly showed colonies exhibiting *B. anthracis* morphology. This observation strongly suggested that an inhibitory compound from the treated wood prevented the growth of *B. anthracis* in liquid culture (where the concentration of the inhibitory compound was the greatest), but the organism flourished once a small amount of the liquid culture was plated out onto nutrient agar. Therefore, the positive control and test coupons of treated wood were indicated as positive for growth in Table 8-4 because the plating step established the presence and viability of *B. anthracis* in the liquid culture.

8.3 Damage to Coupons

No visible damage was observed on the test materials after the 30 min contact time for non-porous materials and 60 min contact time for the porous materials with EasyDECON® 200, with either three or six applications of the decontaminant. The treated wood extracts had a noticeable yellowish hue, probably due to leaching of treatment chemicals from the coupon material.

8.4 Other Factors

8.4.1 Operator Control

On each day of testing, the three components of EasyDECON® 200 were weighed and mixed according to the vendor's explicit instructions, as incorporated into the application procedure in Appendix D. Prior to each application, the EasyDECON® 200 spray nozzle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application, the spray nozzle was removed from the bottle and any residual EasyDECON® 200 was removed by repeated pulls on the trigger of the spray nozzle. All material coupons were oriented horizontally (i.e., lying flat) and stayed in that orientation throughout the entire contact time.

Table 8-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores—EasyDECON® 200

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Brick												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Treated Wood												
Positive Controls[c]	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons[c]	+	+	+	+	+	-	+	+	+	+	+	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
B = Blank (not inoculated with *B. anthracis* (Ames) spores); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = Coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.
[c] Treated wood coupons showed no growth in one-day or seven-day incubation, but showed growth consistent with *B. anthracis* morphology when culture was plated (see text).

All tests were conducted under ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1°C). The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 70%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. Therefore, the testing chamber RH was always ≤ 70% during the decontamination of all test materials with EasyDECON® 200.

8.4.2 Technology Spray Deposition

EasyDECON® 200 was applied according to the procedures included as Appendix D of this report. EasyDECON® 200 was applied from a distance of 30.5 cm to the horizontally-oriented material coupons until the coupons were fully wetted. As described in Section 8.2.1, reapplication of the EasyDECON® 200 was then made on three of the nonporous materials (glass, aluminum, porcelain) at 10 and 20 minutes after the initial application for a total of three applications, and on the other two nonporous materials (stainless steel, granite) at 5, 10, 15, 20, and 25 minutes after the initial application for a total of six applications, within a 30-minute total contact time. Reapplication of the EasyDECON® 200 was made on the porous materials at 10, 20, 30, 40, and 50 minutes after the initial application for a total of six applications within a 60-minute total contact time. After the 30-minute contact time for the nonporous materials or the 60-minute contact time for the porous materials each coupon was placed in the 50 mL conical vial that also served to collect excess decontaminant that may have pooled on the coupon surface.

To assess EasyDECON® 200 deposition, triplicate coupons of each test material were weighed prior to application of the EasyDECON® 200 in the trial runs, and these values were recorded. Then the triplicate coupons were sprayed with EasyDECON® 200 in their horizontal orientation according to the procedures in Appendix D, and each coupon was weighed again after their respective contact times. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of EasyDECON® 200 from each of the test materials is shown in Table 8-5, for the application procedure used with each material in testing. The average deposition values of 0.12 g for glass, aluminum, and porcelain, 0.32 g for stainless steel and granite, and 0.95 g for porous materials were used to estimate the amount of sodium thiosulfate (STS) needed to effectively neutralize the EasyDECON® 200.

8.4.3 Neutralization Methodology

Neutralization of EasyDECON® 200 was achieved with STS. The concentrations of STS tested during the neutralization trial were 0.5, 1.0, and 1.5% in the PBS/Triton® X-100 extraction solution. The results of the neutralization trials are shown for the nonporous materials in Tables 8-6 and 8-7 (three and six applications, respectively), and for the porous materials in Table 8-8. On the basis of these results 1.0% STS was used for neutralization of EasyDECON® 200 in testing with three applications on nonporous materials, and 1.5% STS was used for neutralization of EasyDECON® 200 in testing with six applications, on both porous and nonporous materials.

Table 8-5. Deposition/Runoff Weight of EasyDECON® 200 on Test Materials

Test Material	Average Deposition/ Runoff Weight (g)
Nonporous	
Glass[a]	0.08
Aluminum[a]	0.14
Porcelain[a]	0.13
Average	*0.12*
Stainless Steel[b]	0.27
Granite[b]	0.36
Average	*0.32*
Porous[c]	
Concrete	0.80
Brick	0.87
Asphalt Paving	0.99
Treated Wood	1.27
Butyl Rubber	0.80
Average	*0.95*

[a] These materials tested with three applications, 30-minute contact time.
[b] These materials tested with six applications, 30-minute contact time.
[c] All porous materials tested with six applications, 60-minute contact time.

Table 8-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for EasyDECON® 200 on Nonporous Test Materials: Glass, Aluminum, and Porcelain (3 applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
EasyDECON® 200 + Spores[a]	7.47 x 10^7	0	0
EasyDECON® 200 + PBS + Triton® X-100 + Spores[a,b]	7.47 x 10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	7.47 x 10^7	7.01 x 10^7	100
EasyDECON® 200 + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	7.47 x 10^7	7.73 x 10^7	110.3
EasyDECON® 200 + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	7.47 x 10^7	7.10 x 10^7	101.3
EasyDECON® 200 + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	7.47 x 10^7	6.60 x 10^7	94.1

[a] EasyDECON® 200 volume of 0.12 mL corresponds to mean gravimetric deposition on glass, aluminum, and porcelain, assuming density of 1.0 g/mL.
[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with EasyDECON® 200 = 10.12 mL (10 mL PBS/Triton® X-100/STS + 0.12 mL EasyDECON® 200).

Table 8-7. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for EasyDECON® 200 on Nonporous Test Materials: Stainless Steel and Granite (6 applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
EasyDECON® 200 + Spores[a]	9.30 x 10^7	0	0
EasyDECON® 200 + PBS + Triton® X-100 + Spores[a,b]	9.30 x 10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	9.30 x 10^7	8.49 x 10^7	100
EasyDECON® 200 + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	9.30 x 10^7	8.32 x 10^7	98.1
EasyDECON® 200 + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	9.30 x 10^7	8.44 x 10^7	99.4
EasyDECON® 200 + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	9.30 x 10^7	8.62 x 10^7	101.6

[a] EasyDECON® 200 volume of 0.32 mL corresponds to mean gravimetric deposition on stainless steel and granite, assuming density of 1.0 g/mL.
[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with EasyDECON® 200 = 10.32 mL (10 mL PBS/Triton® X-100/STS + 0.32 mL EasyDECON® 200).

Table 8-8. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for EasyDECON® 200 on Porous Test Materials (6 applications)

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
EasyDECON® 200 + Spores[a]	9.30×10^7	0	0
EasyDECON® 200 + PBS + Triton® X-100 + Spores[a,b]	9.30×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	9.30×10^7	8.85×10^7	100
EasyDECON® 200 + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	9.30×10^7	8.37×10^7	94.7
EasyDECON® 200 + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	9.30×10^7	7.85×10^7	88.8
EasyDECON® 200 + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	9.30×10^7	8.41×10^7	95.0

[a] EasyDECON® 200 volume of 0.95 mL corresponds to mean gravimetric deposition on porous materials, assuming density of 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with EasyDECON® 200 = 10.95 mL (10 mL PBS/Triton® X-100/STS + 0.95 mL EasyDECON® 200).

9.0
Spor-Klenz® RTU Test Results

9.1 QC Results

In testing of Spor-Klenz® RTU, all positive control results were well within the target recovery range of 1 to 150% of the spiked spores. Positive control recovery values on the nonporous materials ranged from 48 to 75%, with the lowest recovery occurring on granite and the highest on anodized aluminum. Positive control recovery values on the porous materials ranged from 17 to 29%, with the lowest recovery occurring on treated wood and the highest on butyl rubber.

In testing of Spor-Klenz® RTU, all procedural and laboratory blanks met the criterion of no observed CFU in quantitative efficacy testing with *B. anthracis*. No growth was observed in the qualitative assessment of residual spores for all procedural and laboratory blanks.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for three days of *B. anthracis* testing were all within that criterion, at 9.30×10^7/coupon, 8.63×10^7/coupon, and 7.80×10^7/coupon, respectively.

9.2 Decontamination Efficacy

The decontamination efficacy of Spor-Klenz® RTU was evaluated for *B. anthracis* (Ames) on ten outdoor material surfaces. The following sections summarize the results found with this decontaminant.

9.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The results for decontamination efficacy of Spor-Klenz® RTU on nonporous and porous materials are shown in Tables 9-1 and 9-2, respectively, and summarized in Table 9-3. The decontamination efficacy of Spor-Klenz® RTU was greater than 7.5 log reduction on porcelain and granite, as shown in Table 9-1. No viable spores were found on any coupons of these two nonporous materials. Efficacy on the other three nonporous materials ranged from 7.17 to 7.36 log reduction. With each of these three materials, viable spores were found on only one of the five test coupons after decontamination.

Table 9-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—Spor-Klenz® RTU on Nonporous Materials (30 minute contact time with one reapplication at 25 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	9.30×10^7	7.74 ± 0.04	59.9 ± 6.0	-
Test Coupons[c]	9.30×10^7	0.46 ± 1.03	0.000045 ± 0.000097	7.28 ± 0.91
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Glass				
Positive Controls	9.30×10^7	7.76 ± 0.06	62.4 ± 9.1	-
Test Coupons	9.30×10^7	0.40 ± 0.90	0.000023 ± 0.000048	7.36 ± 0.79
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Aluminum				
Positive Controls	9.30×10^7	7.84 ± 0.04	75.5 ± 6.4	-
Test Coupons	9.30×10^7	0.67 ± 1.50	0.00050 ± 0.0011	7.17 ± 1.32

Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Porcelain				
Positive Controls	9.30 x 10^7	7.72 ± 0.05	56.4 ± 7.0	-
Test Coupons	9.30 x 10^7	0	0	≥ 7.72 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Granite				
Positive Controls	7.80 x 10^7	7.57 ± 0.07	47.8 ± 7.7	-
Test Coupons	7.80 x 10^7	0	0	≥ 7.57 ± 0.06
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 9-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— Spor-Klenz® RTU on Porous Materials (60 minute contact time with reapplications at 10, 25, 30, and 50 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	8.63 x 10^7	7.19 ± 0.36	23.5 ± 18.3	-
Test Coupons[c]	8.63 x 10^7	6.17 ± 0.21	2.35 ± 1.41	1.02 ± 0.36
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Brick				
Positive Controls	7.80 x 10^7	7.27 ± 0.26	27.3 ± 16.1	-
Test Coupons	7.80 x 10^7	0	0	≥ 7.27 ± 0.22
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Asphalt Paving				
Positive Controls	7.80 x 10^7	7.27 ± 0.14	24.8 ± 8.8	-
Test Coupons	7.80 x 10^7	4.71 ± 0.92	0.25 ± 0.33	2.56 ± 0.81
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Treated Wood				
Positive Controls	8.63 x 10^7	7.05 ± 0.26	17.1 ± 14.7	-
Test Coupons	8.63 x 10^7	0.89 ± 1.25	0.00011 ± 0.00021	6.16 ± 1.12

Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Butyl Rubber				
Positive Controls	8.63 x 10^7	7.39 ± 0.10	29.1 ± 7.3	-
Test Coupons	8.63 x 10^7	0	0	≥ 7.39 ± 0.09
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
"-" Not Applicable.

Table 9-3. Summary of Efficacy Values (Log Reduction) Obtained for Spor-Klenz® RTU

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	7.28
Glass	7.36
Aluminum	7.17
Porcelain	≥ 7.72
Granite	≥ 7.57
Porous	
Concrete	1.02
Brick	≥ 7.27
Asphalt Paving	2.56
Treated Wood	6.16
Butyl Rubber	≥ 7.39

The decontamination efficacy of Spor-Klenz® RTU was greater than 7.27 log reduction on brick and butyl rubber, as shown in Table 9-2. No viable spores were found on any coupons of these two porous materials. Efficacy on unpainted concrete, asphalt paving, and treated wood was approximately 1.02, 2.56, and 6.16 log reduction, respectively, as shown in Table 9-2.

9.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Table 9-4 for coupons spiked with *B. anthracis* (Ames). In this assessment, cultures showing positive growth (*i.e.*, a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. Colonies consistent with the morphology of *B. anthracis* were found only in cultures of coupons inoculated with *B. anthracis*.

The qualitative efficacy results in Table 9-4 are consistent with the quantitative efficacy results summarized in Table 9-3 in that no growth was observed at either one or seven days incubation from the four materials that showed complete inactivation in the quantitative testing (i.e., porcelain, granite, brick, and butyl rubber). Also, the other nonporous materials (stainless steel, glass, and aluminum) each showed growth for *B. anthracis* on the same single test coupon on which viable spores were observed after decontamination in the quantitative testing. The other porous materials (concrete, asphalt, and treated wood) were all strongly positive for growth as shown in Table 9-4, consistent with the relatively low quantitative efficacy values on these materials (Table 9-3). Table 9-4 also shows that all laboratory and procedural blanks were negative for growth.

The same observation noted in previous chapters was made with the treated wood positive control and test coupons. That is, the liquid culture growth assessments for all treated wood coupons were negative (*i.e.*, clear) after both one and seven days' incubation, even though

Table 9-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores—Spor-Klenz® RTU

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	+	-	-[b]	-	-	-	+	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	+	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	-	-	-	-	-	+	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Brick												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Treated Wood												
Positive Controls[c]	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons[c]	+	+	+	+	+	-	+	+	+	+	+	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-

S1 to S5 = Sample 1 to Sample 5.
B = Blank (not inoculated with *B. anthracis* (Ames) spores); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = Coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.
[c] Treated wood coupons showed no growth in one day or seven day incubation, but showed growth consistent with *B. anthracis* morphology when culture was plated (see text).

positive controls had not been decontaminated and the Spor-Klenz® RTU efficacy on the test coupons was incomplete (6.16 log reduction). These negative liquid culture growth assessments were plated on nutrient agar, and all such plates clearly exhibited *B. anthracis* colonies the following day. This observation suggested that an inhibitory compound from the treated wood may have prevented the growth of *B. anthracis* in liquid culture (where the concentration of the inhibitory compound was the greatest), but the organism flourished once a small amount of this liquid culture was streaked out on nutrient agar. Therefore, all treated wood coupons were indicated as positive for growth in Table 9-4 because the plating step established the presence and viability of *B. anthracis* in the liquid culture.

9.3 Damage to Coupons

No visible damage was observed on the test materials after the 30 min contact time for non-porous materials or the 60 min contact time for the porous materials with Spor-Klenz® RTU. The treated wood extracts had a noticeable yellowish hue, probably due to leaching of treatment chemicals from the coupon material.

9.4 Other Factors

9.4.1 Operator Control

On each day of testing, Spor-Klenz® RTU was transferred to a new, handheld, plastic spray bottle. Prior to each application, the Spor-Klenz® RTU spray bottle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application the spray nozzle was removed from the bottle and any residual Spor-Klenz® RTU was removed by repeated pulls on the trigger of the spray nozzle. All coupons were oriented horizontally (i.e., lying flat) and stayed in that orientation throughout the entire contact time.

All tests were conducted at ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1 °C). The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached 70%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. Therefore, the testing chamber was always at ≤ 70% RH during the decontamination of test materials with Spor-Klenz® RTU.

9.4.2 Technology Spray Deposition

Spor-Klenz® RTU was applied according to the procedure included as Appendix E of this report. Specifics of the application procedure (i.e., the schedule of reapplications needed to maintain wetting of the test coupons) were defined in test runs to determine deposition on the test coupons. Spor-Klenz® RTU was applied from a distance of 30.5 cm to the horizontally oriented coupons until the materials were fully wetted. A single reapplication of the Spor-Klenz® RTU was needed to maintain wetting of the nonporous materials at 25 minutes after the initial application, for a total of two applications in the 30 minute total contact time on those materials. Four reapplications of the Spor-Klenz® RTU were needed to maintain wetting on the porous materials at 10, 25, 30, and 50 minutes after the initial application, for a total of five applications in the 60 minute total contact time on those materials. The application procedures thus established were used in the deposition measurements, and in turn were used in all efficacy testing of Spor-Klenz® RTU. After the respective contact times, each material coupon was placed in the 50 mL conical vial that also served to collect excess formulation that may have pooled on its surface.

To assess Spor-Klenz® RTU deposition, triplicate coupons of each test material were weighed prior to application of the Spor-Klenz® RTU in trial runs, and those values were recorded. Then the triplicate coupons were sprayed with Spor-Klenz® RTU in their horizontal orientation according to the procedures established based on Appendix E, and each coupon was weighed again after the respective contact time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the Spor-Klenz® RTU from each of the test materials is shown in Table 9-5. The average deposition amounts of 0.16 and 0.48 g for nonporous and porous materials, respectively, were used to estimate the amount of STS needed to effectively neutralize the Spor-Klenz® RTU.

Table 9-5. Deposition/Runoff Weight of Spor-Klenz® RTU on Test Materials

Test Material	Average Deposition/ Runoff Weight (g)
Nonporous	
Glass	0.12
Aluminum	0.21
Stainless Steel	0.14
Granite	0.12
Porcelain	0.21
Average	*0.16*
Porous	
Concrete	0.22
Brick	0.78
Asphalt Paving	0.20
Treated Wood	0.81
Butyl Rubber	0.37
Average	*0.48*

9.4.3 Neutralization Methodology

Neutralization of Spor-Klenz® RTU was achieved with STS. The concentrations of STS used during the neutralization trials were 0.5, 1.0, and 1.5% in the PBS/Triton® X-100 extraction solution. The results of the neutralization trials are shown in Tables 9-6 and 9-7 for the nonporous and porous materials, respectively. These tables show that in both the nonporous and porous material trials, the action of Spor-Klenz® RTU was inhibited by dilution with PBS/Triton® X-100 extraction solution, substantial recovery of spores was seen with Spor-Klenz® RTU plus PBS solution in the absence of STS (second row of Tables 9-6 and 9-7). This observation implies that partial neutralization of Spor-Klenz® RTU would occur in coupon extraction with the PBS/Triton®X-100 solution. However, added STS was needed to achieve complete neutralization of Spor-Klenz® RTU. On the basis of these trials 0.5% STS was used for neutralization of Spor-Klenz® RTU for both nonporous and porous materials.

Table 9-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Spor-Klenz® RTU on Nonporous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Spor-Klenz® RTU + Spores[a]	7.00 x 10^{7c}	0	0[d]
Spor-Klenz® RTU + PBS + Triton® X-100 + Spores[a,b]	7.00 x 10^{7c}	6.84 x 10^7	97.1[d]
PBS + Triton® X-100 + Spores (Control)[b]	9.07 x 10^7	9.13 x 10^7	100
Spor-Klenz® RTU + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	9.07 x 10^7	9.05 x 10^7	99.1
Spor-Klenz® RTU + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	9.07 x 10^7	1.55 x 10^8	170.1
Spor-Klenz® RTU + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	9.07 x 10^7	9.23 x 10^7	101.1

[a] Spor-Klenz® RTU volume of 0.16 mL corresponds to mean gravimetric deposition on non-porous materials, assuming density of 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Spor-Klenz® RTU = 10.16 mL (10 ml PBS/Triton® X-100/STS + 0.16 mL Spor-Klenz® RTU).

[c] Inadequate number of dilutions prepared in initial trial with 9.07 x 10^7 inoculum; these trials redone, resulting in different inoculum.

[d] Percentage calculated by applying observed spore recovery with 7.00 x 10^7 inoculum to control inoculum of 9.07 x 10^7.

Table 9-7. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Spor-Klenz® RTU on Porous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Spor-Klenz® RTU + Spores[a]	7.00 x 10^{7c}	0	0[d]
Spor-Klenz® RTU + PBS + Triton® X-100 + Spores[a,b]	7.00 x 10^{7c}	1.43 x 10^7	21.7[d]
PBS + Triton® X-100 + Spores (Control)[b]	9.07 x 10^7	8.55 x 10^7	100
Spor-Klenz® RTU + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	9.07x 10^7	9.45 x 10^7	110.4
Spor-Klenz® RTU + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	9.07 x 10^7	8.94 x 10^7	104.6
Spor-Klenz® RTU + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	9.07 x 10^7	8.94 x 10^7	104.6

[a] Spor-Klenz® RTU volume of 0.48 mL corresponds to mean gravimetric deposition on porous materials, assuming density of 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Spor-Klenz® RTU = 10.48 mL (10 ml PBS/Triton® X-100/STS + 0.48 mL Spor-Klenz® RTU).

[c] Inadequate number of dilutions prepared in initial trial with 9.07 x 10^7 inoculum; these trials redone, resulting in different inoculum.

[d] Percentage calculated by applying observed spore recovery with 7.00 x 10^7 inoculum to control inoculum of 9.07 x 10^7.

10.0
Peridox® RTU Test Results

10.1 QC Results

In testing of Peridox® RTU, all positive control recovery results were well within the target range of 1 to 150% of the spiked spores. Positive control recovery values on the nonporous materials ranged from about 6.4 to 76%, with the lowest recovery occurring on stainless steel and the highest on anodized aluminum. The stainless steel recovery of 6.4% was notably lower than in other tests, but recoveries from the materials inoculated at the same time (i.e., granite, brick, and butyl rubber) were not unusually low for those materials, so no systematic error in inoculation is suspected. Positive control recovery values on the porous materials ranged from about 6.3 to 45%, with the lowest recovery occurring on butyl rubber and the highest on asphalt paving.

In quantitative efficacy testing of Peridox® RTU with *B. anthracis*, most procedural and laboratory blanks met the criterion of no observed CFU. However, all procedural and laboratory blanks of the four materials tested on the third day of Peridox® RTU testing (i.e., stainless steel, granite, brick, and butyl rubber) were found to produce CFU of characteristic *B. anthracis* morphology upon streak plating. An investigation of test procedures disclosed that in that test, a laboratory trainee mistakenly handled positive control coupons using a laboratory forceps and then used the same forceps to handle the blank coupons, thereby contaminating the extraction solutions into which the blank coupons were placed. This procedure violated established testing procedures, which call for handling blank coupons before handling any inoculated coupons to avoid contamination. The observed contamination had no impact on the log reduction determined for Peridox® RTU on these test materials, as test coupons were handled properly. However, a test/QA plan deviation was prepared and placed in the project files to document this departure from test procedures.

No growth was observed in the qualitative assessment of residual spores for all procedural and laboratory blanks.

Spike control samples were taken from the spore suspension on each day of testing, and serially diluted, nutrient plated, and counted to establish the spore density used to spike the coupons. This process takes approximately 24 hours, so the spore density is known after completion of each day's testing. The target criterion is to maintain a spore suspension density of 1×10^9/mL (± 25%), leading to a spike of 1×10^8 spores (± 25%) on each test coupon. The actual spike values for three days of *B. anthracis* testing were all within that criterion, at 8.83×10^7/coupon, 8.87×10^7/coupon and 8.20×10^7/coupon, respectively.

10.2 Decontamination Efficacy

The decontamination efficacy of Peridox® RTU was evaluated for *B. anthracis* (Ames) on 10 outdoor material surfaces. The following sections summarize the results found with this decontaminant.

10.2.1 Quantitative Assessment of the Log Reduction of Viable Organisms

The results for decontamination efficacy of Peridox® RTU on nonporous and porous materials are shown in Tables 10-1 and 10-2, respectively, and summarized in Table 10-3. The contaminated blank coupons of four materials noted in Section 10.1 are identified in Tables 10-1 and 10-2, and denoted by a footnote in each table.

The decontamination efficacy of Peridox® RTU for *B. anthracis* was ≥ 6.69 log reduction on stainless steel, and greater than 7.42 log reduction on the other nonporous materials, as shown in Table 10-1. No viable spores were found on any coupons of the nonporous materials decontaminated with Peridox® RTU. The decontamination efficacy of Peridox® RTU was not as consistent on the porous materials, as shown in Table 10-2. Log reductions on treated wood and butyl rubber were ≥ 6.99 and ≥ 6.65 logs, respectively, and no viable spores were found on any coupons of those materials decontaminated with Peridox® RTU. Efficacy on asphalt paving coupons was similarly high (7.22 log reduction), although viable spores were recovered from one decontaminated asphalt coupon. Unpainted concrete and brick had log reductions of approximately 1.39 and 3.81, respectively, and viable spores were recovered from all decontaminated coupons of those materials.

10.2.2 Qualitative Assessment of Residual Spores

Results from the liquid culture growth assessment of coupons at one and seven days post-decontamination are provided in Table 10-4 for coupons spiked with *B. anthracis* (Ames). In this assessment, cultures showing positive growth (*i.e.*, a cloudy growth medium) were subjected to streak plating and the identity of the growing organism was checked by colony morphology. Only colonies consistent with the morphology of *B. anthracis* were found in cultures of coupons inoculated with *B. anthracis*.

The qualitative efficacy results in Table 10-4 are largely consistent with the quantitative efficacy results summarized in Table 10-3 in that no growth was observed at either one or seven days incubation from the five nonporous materials that showed complete inactivation in the quantitative testing, or from the porous material treated wood, which also showed complete inactivation in the quantitative testing. All test coupons of concrete and brick showed positive growth at both one and seven days' incubation, consistent with the relatively low quantitative efficacy found with those materials. Two test coupons of asphalt paving showed positive growth, whereas viable spores were recovered from only one coupon in the quantitative testing.

The exception in terms of consistency relative to the quantitative results was for butyl rubber, in that three test coupons of that material showed positive growth at both one and seven days incubation, although no viable spores were recovered from that material in the quantitative

Table 10-1. Inactivation of *Bacillus anthracis* (Ames) Spores[a]—Peridox® RTU on Nonporous Materials (30 minute contact time with re-applications at 10 and 25 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Stainless Steel				
Positive Controls[b]	8.20×10^7	6.69 ± 0.17	6.4 ± 2.7	-
Test Coupons[c]	8.20×10^7	0	0	≥ 6.69 ± 0.15
Laboratory Blank[d]	0	2.01[f]	0.0001	-
Procedural Blank[e]	0	3.42[f]	0.003	-
Glass				
Positive Controls	8.83×10^7	7.76 ± 0.03	65.3 ± 5.1	-
Test Coupons	8.83×10^7	0	0	≥ 7.76 ± 0.03
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Aluminum				
Positive Controls	8.83×10^7	7.82 ± 0.05	75.8 ± 9.2	-
Test Coupons	8.83×10^7	0	0	≥ 7.82 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Porcelain				
Positive Controls	8.83×10^7	7.71 ± 0.05	57.8 ± 7.5	-
Test Coupons	8.83×10^7	0	0	≥ 7.71 ± 0.05
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Granite				
Positive Controls	8.20×10^7	7.42 ± 0.12	32.7 ± 8.6	-
Test Coupons	8.20×10^7	0	0	≥ 7.42 ± 0.11
Laboratory Blank	0	3.54[f]	0.004	-
Procedural Blank	0	2.31[f]	0.0003	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).

CI = Confidence interval (± 1.96 × SE).

[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).

[c] Test Coupons = Inoculated, decontaminated coupons.

[d] Laboratory Blank = Not inoculated, not decontaminated coupon.

[e] Procedural Blank = Not inoculated, decontaminated coupon.

[f] Blank coupons contaminated after testing due to improper handling procedure; see text.

"-" Not Applicable.

Table 10-2. Inactivation of *Bacillus anthracis* (Ames) Spores[a]— Peridox® RTU on Porous Materials (60 minute contact time with re-applications at 10, 20, 30, 40, and 50 minutes)

Test Material	Inoculum (CFU)	Mean of Logs of Observed CFU	Mean % Recovery	Decontamination Efficacy ± CI
Concrete				
Positive Controls[b]	8.87 x 10^7	7.10 ± 0.07	14.2 ± 2.4	-
Test Coupons[c]	8.87 x 10^7	5.71 ± 0.16	0.61 ± 0.26	1.39 ± 0.15
Laboratory Blank[d]	0	0	0	-
Procedural Blank[e]	0	0	0	-
Brick				
Positive Controls	8.20 x 10^7	6.78 ± 0.21	7.9 ± 3.3	-
Test Coupons	8.20 x 10^7	2.97 ± 1.11	0.0087 ± 0.017	3.81 ± 0.99
Laboratory Blank	0	4.13[f]	0.016	-
Procedural Blank	0	4.17[f]	0.018	-
Asphalt Paving				
Positive Controls	8.87 x 10^7	7.59 ± 0.04	44.5 ± 4.4	-
Test Coupons	8.87 x 10^7	0.37 ± 0.83	0.000017 ± 0.000035	7.22 ± 0.73
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Treated Wood				
Positive Controls	8.87 x 10^7	6.99 ± 0.04	11.1 ± 1.0	-
Test Coupons	8.87 x 10^7	0	0	≥ 6.99 ± 0.03
Laboratory Blank	0	0	0	-
Procedural Blank	0	0	0	-
Butyl Rubber				
Positive Controls	8.20 x 10^7	6.65 ± 0.28	6.3 ± 3.4	-
Test Coupons	8.20 x 10^7	0	0	≥ 6.65 ± 0.25
Laboratory Blank	0	2.15[f]	0.0002	-
Procedural Blank	0	2.55[f]	0.0004	-

[a] Data are expressed as the mean (± SD) of the logs of the number of spores (CFU) observed on five individual coupons, the mean percent recovery on those five coupons, and decontamination efficacy (log reduction).
CI = Confidence interval (± 1.96 × SE).
[b] Positive Controls = Inoculated, not decontaminated coupons (sprayed with SFW).
[c] Test Coupons = Inoculated, decontaminated coupons.
[d] Laboratory Blank = Not inoculated, not decontaminated coupon.
[e] Procedural Blank = Not inoculated, decontaminated coupon.
[f] Blank coupons contaminated after testing due to improper handling procedure; see text.
"-" Not Applicable.

Table 10-3. Summary of Efficacy Values (Log Reduction) Obtained for Peridox® RTU

Test Material	Efficacy for *B. anthracis* (Ames)
Nonporous	
Stainless Steel	≥ 6.69
Glass	≥ 7.76
Aluminum	≥ 7.82
Porcelain	≥ 7.71
Granite	≥ 7.42
Porous	
Concrete	1.39
Brick	3.81
Asphalt Paving	7.22
Treated Wood	≥ 6.99
Butyl Rubber	≥ 6.65

efficacy testing. Growth in the qualitative testing was also observed with a few coupons of porcelain, but the morphology of the resulting CFU upon plating was clearly not consistent with the morphology of *B. anthracis*, so all porcelain coupons are shown as negative for growth in Table 10-4. The laboratory and procedural blanks were all negative for growth.

10.3 Damage to Coupons

No visible damage was observed on the test materials after the 30 minute contact time on non-porous materials and the 60 minute contact time on the porous materials with Peridox® RTU. The treated wood extracts had a yellowish hue, probably due to leaching of treatment chemicals from the coupon material.

10.4 Other Factors

10.4.1 Operator Control

On each day of testing, Peridox® RTU was transferred to a new, handheld, plastic spray-bottle. Prior to each application, the Peridox® RTU spray-bottle was primed by repeatedly spraying into an absorbent cloth to clear any air bubbles that may have formed between applications. After each application the spray nozzle was removed from the bottle and any residual Peridox® RTU was removed by repeated pulls on the trigger of the spray nozzle. All coupons were oriented horizontally (i.e., lying flat) and stayed in that orientation throughout the entire contact time.

All tests were conducted at ambient conditions inside a climate-controlled laboratory. The temperature inside the test chamber was equilibrated to the ambient laboratory temperature, measured to be 22 °C (± 1°C). The RH inside the test chamber was monitored with a NIST-traceable hygrometer. Whenever the RH reached

Table 10-4. Liquid Culture Assessment of Extracts from Coupons Inoculated with *Bacillus anthracis* (Ames) Spores—Peridox® RTU

Test Material	Day 1						Day 7					
	S1	S2	S3	S4	S5	B	S1	S2	S3	S4	S5	B
Stainless Steel												
Positive Controls	+	+	+	+	+	-[a]	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-[b]	-	-	-	-	-	-
Glass												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Aluminum												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Porcelain												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Granite												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Concrete												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-

Brick												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	+	+	+	+	-	+	+	+	+	+	-
Asphalt Paving												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	+	+	-	-	-	-	+	+	-	-	-
Treated Wood												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	-	-	-	-	-	-	-	-	-	-	-	-
Butyl Rubber												
Positive Controls	+	+	+	+	+	-	+	+	+	+	+	-
Test Coupons	+	-	+	+	-	-	+	-	+	+	-	-

S1 to S5 = Sample 1 to Sample 5.
B = Blank (not inoculated with *B. anthracis* (Ames) spores); a = laboratory blank, b = procedural blank.
Positive controls = Coupons inoculated with *B. anthracis* (Ames) spores, but not subjected to decontamination.
Test coupons = coupons inoculated with *B. anthracis* (Ames) spores, and subjected to decontamination.
"+" = growth; "-" = no growth.

70%, the dehumidification system attached to the testing chamber was actuated until the RH dropped below 70%. Therefore, the testing chamber humidity was always ≤ 70% RH during the decontamination of test materials with Peridox® RTU.

10.4.2 Technology Spray Deposition

Peridox® RTU was applied according to the procedure included as Appendix F of this report. Peridox® RTU was applied from a distance of 30.5 cm to the horizontally-oriented materials until the materials were fully wetted. With nonporous materials two reapplications of Peridox® RTU were needed to maintain wetting, at 10 and 25 minutes after the initial application, for a total of three applications in the 30 minute total contact time on those materials. With porous materials, five reapplications were needed at 10, 20, 30, 40, and 50 minutes, for a total of six total applications in the 60 minute total contact time on those materials. These application procedures were used in the deposition measurements, and in turn were used in all efficacy testing of Peridox® RTU. After the respective contact times, each material coupon was placed in the 50 mL conical vial that also served to collect excess formulation that may have pooled on the coupon surface.

To assess Peridox® RTU deposition, triplicate coupons of each test material were weighed prior to application of the Peridox® RTU in the trial runs, and those values were recorded. Then the triplicate coupons were sprayed with Peridox® RTU until fully wetted in their horizontal orientation according to the procedures established based on Appendix F, and each coupon was weighed again after the respective contact time. The pre-application weights were then subtracted from the post-application weights, and that difference was added to the weight of decontaminant runoff captured separately from each coupon. The average deposition/runoff weight of the Peridox® RTU from each of the test materials is shown in Table 10-5. The average deposition amounts of 0.30 and 0.64 g for nonporous and porous materials, respectively, were used to estimate the amount of STS needed to effectively neutralize the Peridox® RTU.

10.4.3 Neutralization Methodology

Neutralization of Peridox® RTU was achieved with STS. The concentrations of STS used during the neutralization panel were 0.5, 1.0, and 1.5% in the PBS/Triton® X-100 extraction solution. The results of the neutralization panel are shown in Tables 10-6 and 10-7 for the nonporous and porous materials, respectively

Table 10-6 shows that in the nonporous material trial the action of Peridox® RTU was inhibited by dilution with PBS/Triton® X-100 extraction solution, as substantial recovery of spores was seen with Peridox® RTU plus extraction solution in the absence of STS (second row of Table 10-6). This observation implies that partial neutralization of Peridox® RTU would occur in extraction of nonporous material coupons with the PBS/Triton® X-100 solution. However, added STS was needed to achieve complete neutralization of Peridox® RTU with those coupons. This behavior was not observed in the porous material neutralization trial (Table 10-7), presumably because of the larger quantity of Peridox® RTU used with those materials. On the basis of these trials, 1.0% STS was used for neutralization of Peridox® RTU for the nonporous materials and 1.5% STS was used for neutralization with porous materials.

Table 10-5. Deposition/Runoff Weight of Peridox® RTU on Test Materials

Test Material	Average Deposition/ Runoff Weight (g)
Nonporous	
Glass	0.25
Aluminum	0.34
Stainless Steel	0.36
Granite	0.26
Porcelain	0.27
Average	*0.30*
Porous	
Concrete	0.33
Brick	0.96
Asphalt Paving	0.39
Treated Wood	1.08
Butyl Rubber	0.46
Average	*0.64*

Table 10-6. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Peridox® RTU on Nonporous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Peridox® RTU + Spores[a]	7.73×10^7	0	0
Peridox® RTU + PBS + Triton® X-100 + Spores[a,b]	7.73×10^7	3.01×10^7	45.1
PBS + Triton® X-100 + Spores (Control)[b]	7.73×10^7	6.67×10^7	100
Peridox® RTU + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	7.73×10^7	5.81×10^7	87.2
Peridox® RTU + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	7.73×10^7	5.93×10^8	88.9
Peridox® RTU + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	7.73×10^7	5.88×10^7	88.3

[a] Peridox® RTU volume of 0.30 mL corresponds to mean gravimetric deposition on nonporous materials, assuming density of 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Peridox® RTU = 10.30 mL (10 mL PBS/Triton® X-100/STS + 0.30 mL Peridox® RTU).

Table 10-7. Neutralization Testing with *Bacillus anthracis* (Ames) Spores for Peridox® RTU on Porous Test Materials

Treatment	Inoculum (CFU)	Total Observed (CFU)	% of Control
Peridox® RTU + Spores[a]	7.73×10^7	0	0
Peridox® RTU + PBS + Triton® X-100 + Spores[a,b]	7.73×10^7	0	0
PBS + Triton® X-100 + Spores (Control)[b]	7.73×10^7	5.77×10^7	100
Peridox® RTU + PBS + Triton® X-100 + 0.5% STS + Spores[a,b]	7.73×10^7	8.97×10^6	15.6
Peridox® RTU + PBS + Triton® X-100 + 1.0% STS + Spores[a,b]	7.73×10^7	5.71×10^7	99.0
Peridox® RTU + PBS + Triton® X-100 + 1.5% STS + Spores[a,b]	7.73×10^7	5.87×10^7	101.9

[a] Peridox® RTU volume of 0.64 mL corresponds to mean gravimetric deposition on non-porous materials, assuming density of 1.0 g/mL.

[b] 10 mL Volume of PBS includes 0.1% of Triton® X-100 surfactant and indicated % of STS; total volume for all samples with Peridox® RTU = 10.64 mL (10 mLPBS/Triton® X-100/STS + 0.64 mL Peridox® RTU).

11.0 Performance Summary

11.1 pH-Amended Bleach Results

- The quantitative efficacy of pH-amended bleach for *B. anthracis* was ≥ 7.62 log reduction on all five nonporous materials and ≥ 6.91 log reduction on the porous materials, brick and butyl rubber. On those seven materials inactivation of *B. anthracis* was complete; i.e., no viable spores were found on any decontaminated coupons. Quantitative efficacy was 6.27 log reduction on concrete, 3.60 log reduction on asphalt paving, and 1.90 log reduction on treated wood.
- Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated test coupons of any materials except for asphalt paving and treated wood after one and seven days incubation. Morphological analysis was consistent with the growth observed being only *B. anthracis*.
- No visible damage was observed on any of the test materials after the 60 minute contact time with pH-amended bleach in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

11.2 CASCAD™ SDF Results

- The quantitative efficacy of CASCAD™ SDF for *B. anthracis* was ≥ 6.80 log reduction on all ten materials. On all materials inactivation of *B. anthracis* was complete, i.e., no viable spores were found on any decontaminated coupons.
- Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated test coupons of any materials after one and seven days incubation.
- No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with CASCAD™ SDF in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

11.3 Decon Green Results

- The quantitative efficacy of Decon Green for *B. anthracis* was ≥ 7.32 log reduction on all five nonporous materials, and was ≥ 7.25 and ≥ 6.94 log reduction, respectively, on the porous materials brick and butyl rubber. No viable spores were found on any of these seven test materials after decontamination with Decon Green. Efficacy on concrete, asphalt, and treated wood was lower, with 4.00, 2.97, and 1.91 log reductions, respectively.
- Qualitative efficacy results were consistent with quantitative efficacy results, in that no growth was seen with decontaminated test coupons of seven of the ten test materials after one and seven days incubation. The decontaminated coupons of concrete, asphalt, and treated wood all were positive for growth at both one and seven days incubation. Morphological analysis was consistent with the growth observed being only *B. anthracis*.
- No visible damage was observed on any of the test materials after the 60 minute contact time with Decon Green in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

11.4 EasyDECON® 200 Results

- The quantitative efficacy of EasyDECON® 200 for *B. anthracis* was ≥7.51 log reduction on all five nonporous materials, and ≥6.99 log reduction on the porous materials concrete, brick, and butyl rubber. No viable spores were found on decontaminated coupons of those eight materials. Efficacy on asphalt paving and treated wood was approximately 1.63 and 0.82 log reduction, respectively.
- Qualitative efficacy results were consistent with the quantitative results, in that no growth was seen with decontaminated test coupons of eight of the ten test materials after one and seven days incubation. All decontaminated coupons of asphalt and treated wood were positive for growth at both one and seven days incubation. Morphological analysis was consistent with the growth observed being only *B. anthracis*.
- No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with EasyDECON® 200 in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

11.5 Spor-Klenz® RTU Results

- The quantitative efficacy of Spor-Klenz® RTU for *B. anthracis* was ≥ 7.57 log reduction on the nonporous materials porcelain and granite, and ≥ 7.27 log reduction on the porous materials brick and butyl rubber. No viable spores were found on decontaminated coupons of those four materials.

Efficacy was also relatively high on stainless steel, glass, and aluminum (approximately 7.28, 7.36, and 7.17 log reduction, respectively), but small numbers of viable spores were found on one test coupon of each of these materials after decontamination. On concrete, asphalt paving, and treated wood efficacy was approximately 1.02, 2.56, and 6.16 log reduction, respectively.

- Qualitative efficacy results were consistent with the quantitative results, in that no growth was seen with decontaminated test coupons of four of the ten test materials. Growth was seen with one decontaminated test coupon each of stainless steel, glass, and aluminum. All decontaminated test coupons of concrete, asphalt, and treated wood were positive for growth at both one and seven days incubation. Morphological analysis was consistent with the growth observed being only *B. anthracis*.
- No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with Spor-Klenz® RTU in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

11.6 Peridox® RTU Results

- The quantitative efficacy of Peridox® RTU for *B. anthracis* was ≥ 6.65 log reduction on all the nonporous materials and on the porous materials treated wood and butyl rubber. No viable spores were found on decontaminated coupons of any of these seven materials. Efficacy was also relatively high (7.22 log reduction) on asphalt paving, although a small number of viable spores were found on one test coupon of that material after decontamination. The efficacy of Peridox RTU on concrete and brick was relatively low, at 1.39 and 3.81 log reduction, respectively.
- Qualitative efficacy results were largely consistent with the quantitative results, in that no growth was seen with decontaminated test coupons of the five nonporous materials and of the porous material treated wood. However, three test coupons of butyl rubber showed positive growth after both one and seven days incubation, although no viable spores had been found in the quantitative efficacy testing with this material. All decontaminated coupons of unpainted concrete and brick, and two coupons of asphalt paving, were positive for growth at both one and seven days' incubation. Morphological analysis was consistent with the growth observed being *B. anthracis*.
- No visible damage was observed on any of the test materials after the 30 or 60 minute contact times with Peridox® RTU in the quantitative efficacy testing, or seven days later after completion of the qualitative assessment of residual spores.

Appendix A
Preparation and Application of pH-Amended Bleach

General Description

For testing of efficacy against *B. anthracis* on outdoor materials, pH-amended bleach consists of a specialized germicidal bleach formulation (Clorox® Commercial Solutions Ultra Clorox® Germicidal Bleach), which is diluted in cell-culture grade sterile filtered water (SFW) and has its pH adjusted by addition of a small amount of acetic acid. Specifically, Ultra Clorox® Germicidal Bleach contains a total of about 6.15% by weight of sodium hypochlorite (NaOCl) in aqueous solution. The recipe for preparation of pH-amended bleach for use as a decontaminant is as follows:

- Prepare 5% acetic acid solution by diluting 50 mL of glacial acetic acid up to 1 L with SFW in a volumetric flask.
- Mix 9.4 parts SFW, 1 part Ultra Clorox® Germicidal Bleach, and 1 part 5% acetic acid. The resulting solution will have a mean pH of about 6.8 and a mean total chlorine content of about 6,200 ppm.

The active decontaminating agents in this solution are hypochlorite (OCl^-) and hypochlorous acid. The effectiveness of bleach as a biological decontaminant is widely known, and in particular the vendor indicates that Ultra Clorox® Germicidal Bleach is the only product registered with U.S. EPA as effective in killing *Clostridium difficile* bacteria.

In previous testing of pH-amended bleach as a decontaminant, neutralization of the bleach solution was achieved using sodium thiosulfate (STS). Based on the chemical composition of the pH-amended bleach, the amount of that solution (0.325 mL) retained or run off from a test coupon with a specified 10-second application period, and the use of 10 mL of an extraction solution containing phosphate-buffered saline (PBS) + 0.1% Triton® X-100, it was determined that an STS concentration of 0.086% in the extraction solution was optimal for neutralizing the pH-amended bleach. The application equipment and procedures used in this evaluation differed from those used in previous testing, so the determination of the neutralization procedure was repeated to establish neutralization conditions appropriate for this evaluation.

Application Procedure for Testing

Based on previous test results with pH-amended bleach, and considering the surface materials to be used in this testing, an application procedure for use in testing was developed. The intent of this procedure was to employ conventional and readily available equipment in a relatively simple application process. Trial runs were conducted to establish the appropriate concentration of STS for neutralization of the pH-amended bleach.

The test coupon materials used with pH-amended bleach included the nonporous materials steel, glass, aluminum, porcelain, and granite, and the porous materials concrete, brick, asphalt paving, treated wood, and butyl rubber.

The pH-amended bleach was prepared fresh shortly before use on each day of testing, as described above. The pH of the solution was measured and recorded as part of the test data. A new noncorroding garden pump sprayer was used to apply the solution of pH-amended bleach to the test coupon surfaces. An identical sprayer was used to apply SFW to positive control test coupons. Each sprayer was fitted with a pressure gauge to indicate the internal delivery pressure of the sprayer. The internal pressure of each sprayer was maintained in a normal range for use (i.e., 4 to 6 psi) throughout all applications. Based on laboratory tests, such a range of pressures produces a stable spray suitable for application on the scale of coupon testing. The step-by-step application procedure was:

- Apply the pH-amended bleach solution to the test coupons (or SFW to the positive control coupons) from a distance of about one foot (30.5 cm) using the sprayer at a delivery pressure within the specified range, until the test coupon surfaces are fully wetted by the solution.
- Reapply the solution three times, i.e., at 15 minutes after the first application, 30 minutes after the first application, and 45 minutes after the first application.
- If necessary, pump up the pressure in the sprayer before application to maintain pressure within the specified range.
- When 60 minutes have elapsed since the start of the first application, place the coupons into the extraction solution (containing the neutralization agent) along with any collected runoff of pH-amended bleach.

Appendix B
Preparation and Application of CASCAD™ SDF

General Description

CASCAD™ Surface Decontamination Foam (SDF) uses two liquid solutions (A and B) which react to form a foam as they are mixed upon release from the application device. These two solutions are made from three separate reagents, having chemical composition as follows:

- GPA-2100 (decontaminant) – solid reagent in powder form consisting of dichloroisocyanuric acid sodium salt, 70 to 100% by weight;
- GPB-2100 (buffer) – solid reagent in powder form consisting of sodium tetraborate 10 to 30%, sodium hydroxide 1 to 5 %, and sodium carbonate 40 to 65% by weight;
- GCE-2000 (surfactant) – liquid reagent consisting of sodium myristyl sulfate 10 to 30%, sodium (C_{14} to C_{16}) olefin sulphonate 10 to 30%, ethanol denatured 3 to 9%, alcohols (C_{10} to C_{16}) 5-10%, sodium sulfate 3 to 7%, sodium xylene sulfonate 1 to 5%, and a proprietary mixture of sodium and ammonium salts along with water and co-solvent >9% by weight.

The A and B solutions are prepared from these reagents by the following procedure:

1. Make solution A by adding 31.2 grams (four 7.8 gram packets) of GPA-2100 to 250 mL of water in a graduated cylinder, and then dilute with SFW to 300 mL.
2. Mix with a micro stir bar until dissolved.
3. Make solution B by adding 7.2 grams (four 1.8 gram packets) of GPB-2100 to 250 mL of SFW in a graduated cylinder.
4. Mix with a micro stir bar until dissolved.
5. Add 18 mL (four 4.5 mL packets) of GCE-2000 to the solution from Step 4, mix, and then dilute with SFW up to 300 mL final volume.

For use on the small scale needed for testing, a manual spray application bottle (the 600 mL Hand Held Decontamination System) has been developed by Allen-Vanguard that draws solutions A and B from separate compartments and delivers them as a foam through a single spray head. To fill and operate the Hand Held System, follow these steps:

1. Pull the Locking Lever on the front of the bottle housing forward and lift to open the housing and expose the solution bottles, which are labeled "A" and "B".
2. With the housing opened remove the caps (turn counter clockwise) and pull out the solution suction lines from the solution bottles.
3. With the caps and suction lines removed from both the "A" and "B" solution bottles:
   a. Pour solution A into the bottle labeled "A", and pour solution B into the bottle labeled "B".
   b Assure that both bottles are seated in the housing with the "B" bottle at the front.
   c. Place the suction lines back into the "A" and "B" bottles and tighten both the "A" and "B" caps by turning them in a clockwise direction.
4. Hold the suction line up with one hand while closing the top of the housing with the other hand. Make certain that the Locking Lever snaps into its recess when the housing top closes The suction line may be pinched closed if this procedure is not followed correctly; openness of the suction line can be checked by looking through the housing and checking the suction line.
5. To use the 600 mL Hand Held Decontamination System, grasp the neck of the housing with your dominant hand and place the finger of this hand on the trigger of the foam nozzle. Aim the tip of the foam nozzle in the direction of the area to be decontaminated and pump the trigger. The trigger may have to be squeezed three or four times to evacuate the air in the suction line before foam is discharged.

Application Procedure for Testing

CASCAD™ SDF was applied to test coupons using the vendor-developed dual spray applicator. In previous testing, neutralization of the CASCAD™ SDF was achieved by addition of 0.5% sodium thiosulfate (STS) to the extraction solution. Trial runs were conducted before testing to establish the appropriate STS concentration for neutralization of the applied CASCAD™ SDF.

The step-by-step application procedure for testing was as indicated below. Note that the procedure for porous materials differed from that for nonporous materials. All test coupons were oriented horizontally (i.e., lying flat) for testing.

- Follow the instructions provided above for preparation of the reagent solutions and loading of the manual spray applicator.
- Squeeze the trigger of the applicator head a few times while pointing the applicator into a laboratory sink or other waste container, until any air is cleared from the applicator and CASCAD™ SDF is delivered from the applicator as a foam.
- Apply the CASCAD™ SDF to the test coupons using the manual applicator from a distance of about one foot (30.5 cm) while moving the nozzle, until the test coupons are entirely covered with no less than one (1) centimeter (3/8”) deep foam.

For **nonporous** coupon materials (glass, steel, aluminum, porcelain, granite):

- Allow the foam to remain on the coupons for 30 minutes. Do not re-apply.
- When 30 minutes have elapsed since the application, place each coupon into the extraction solution (containing the STS neutralization agent) along with any CASCAD™ SDF accumulated on the coupon.

For **porous** coupon materials (concrete, brick, asphalt paving, treated wood, rubber):

- Allow the foam to remain on the coupons for 30 minutes.
- Reapply more CASCAD™ SDF and allow the foam to remain on the coupons for an additional 30 minutes.
- When a total of 60 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the STS neutralization agent) along with any CASCAD™ SDF accumulated on the coupon.

After use, empty and clean the manual spray applicator according to the instructions below.

Cleaning the Hand Held Decontamination System

Clean the CASCAD™ SDF system after use by the following procedure.

1. Dump any remaining decontamination solution from both the “A” and “B” bottles and dispose of the solutions following appropriate waste disposal procedures.
2. Thoroughly rinse both bottles with SFW, and then fill each bottle with clean water.
3. Place the filled bottles back into the housing, insert the suction lines, and close the housing.
4. Pump the trigger until the suction lines and foam nozzle are free from the decontamination solution.
5. Flush the interior and the exterior of the housing, and the caps used while mixing the solution, thoroughly with SFW.

Appendix C
Preparation and Application of Decon Green

General Description

Decon Green is a hydrogen peroxide-based decontaminant designed for biological, chemical, and radiological efficacy. Decon Green consists of a three-part formula which is mixed just prior to use. “Part A” contains surfactants and solvents which impart surface cleaning and penetration ability; “Part B” is aqueous 35 % hydrogen peroxide (H_2O_2), the active ingredient; “Part C” contains activators and buffers. The specific chemical components of each solution are as follows:

- Part A – propylene carbonate ($C_4H_6O_3$) + Triton® X-100 + propylene glycol (1,2-propanediol, $C_3H_8O_2$).
- Part B – hydrogen peroxide 35% aqueous solution.
- Part C – aqueous solution of potassium bicarbonate (potassium hydrogen carbonate, $KHCO_3$) + potassium citrate monohydrate ($C_6H_5K_3O_7{\bullet}H_2O$) + potassium molybdate (K_2MoO_4) + propylene glycol.

These three parts of Decon Green are packaged in three corresponding separate containers. To mix Decon Green, the contents of containers B and C are added to the contents of container A. It is important not to mix the contents of containers B and C without first adding them to the contents of container A, as excessive heating may result. The final solution of Decon Green has a pH of about 8 and a density of approximately 1.1 g/mL.

Sodium thiosulfate (STS) was used to stop the action of Decon Green so that efficacy could be determined. Trial runs were conducted before efficacy testing to establish the appropriate concentration of STS for neutralization of Decon Green.

Application Procedure for Testing

An application procedure for use of Decon Green in testing was developed based on information provided by the manufacturer of the product. The aim is to use a relatively simple application process that is likely to be effective when carried out with conventional and readily available equipment.

Decon Green was applied to test coupons as a liquid solution using a hand-held plastic spray bottle. A similar bottle was used to apply deionized (DI) water to positive control test coupons. The step-by-step application procedure was as follows:

- Apply the Decon Green solution to the test coupons (or SFW to the positive control coupons) from a distance of 30.5 cm using the handheld spray bottle, until all test coupon surfaces are fully wetted by the solution.
- When 30 minutes have elapsed since the first application, re-apply Decon Green to all test coupons.
- When 60 minutes total contact time has elapsed since the first application of Decon Green, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any Decon Green solution pooled on the test coupon.

Appendix D
Preparation and Application of EasyDECON® 200

General Description

EasyDECON® 200 is a liquid decontaminant consisting of a three-part formula which is mixed just prior to use. The specific chemical components of each solution are as follows:

- Part One – quaternary ammonium compounds and benzyl-C_{12} to -C_{16} alkyl dimethyl chlorides, 5.5 to 6.5 % aqueous solution;
- Part Two – hydrogen peroxide < 8% aqueous solution;
- Part Three – diacetin (glycerol diacetate; 1,2,3-propanetriol-1,3-diacetate), 30 to 60% aqueous solution.

These three parts of EasyDECON® 200 are packaged in three corresponding separate containers labeled "Part One," "Part Two," and "Part Three,", premeasured and ready to mix. To prepare EasyDECON® 200 in any amount the required proportions by weight are 49% of Part One and 49% of Part Two mixed in a clean container, and then 2% of Part Three is added and all three components mixed thoroughly. The final solution of EasyDECON® 200 has a pH of about 9.6 to 9.9 and a density of approximately 1.08 g/mL.

Sodium thiosulfate (STS) was used to stop the action of EasyDECON® 200 so that efficacy could be determined. Trial runs were conducted before efficacy testing to establish the appropriate concentration of STS for neutralization of EasyDECON® 200.

Application Procedure for Testing

An application procedure for use of EasyDECON® 200 in testing was developed based on information provided by the vendor. The aim is to use a relatively simple application process that is likely to be effective when carried out with conventional and readily available equipment.

EasyDECON® 200 was applied to test coupons as a liquid solution using a hand-held plastic spray bottle. A similar bottle was used to apply SFW to positive control test coupons. A target application rate of 0.12 to 0.14 g/cm^2 (0.11 to 0.13 mL/cm^2) is recommended by the vendor. This application rate is relatively large, being equivalent to approximately 1.7 to 2.0 g (1.6 to 1.9 mL) applied to a 1.9 × 7.5 cm test coupon. The step-by-step application procedures as indicated below were designed to achieve this target application rate. Note that the application procedure for porous materials differed from that for nonporous materials.

Primary Procedures:

- Apply the EasyDECON® 200 solution to the test coupons (or SFW to the positive control coupons) from a distance of 30.5 cm using the handheld spray bottle, until all test coupon surfaces are fully wetted by the solution.

For nonporous materials (steel, aluminum, glass, porcelain, granite):

- Reapply the EasyDECON® 200 10 minutes after the first application, and again 20 minutes after the first application. Perform additional applications as needed if the test coupon surfaces become dry.
- When 30 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any EasyDECON® 200 solution pooled on the test coupon.

For porous materials (concrete, brick, asphalt paving, treated wood, butyl rubber):

- Reapply the EasyDECON® 200 20 minutes after the first application, and again 40 minutes after the first application. Perform additional applications as needed if the test coupon surfaces become dry.
- When 60 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any EasyDECON® 200 solution pooled on the test coupon.

If the actual application rate of EasyDECON® 200 fell short of the target rate when the primary procedures above are used, the alternate procedures below were used. These alternate procedures relied on six applications of EasyDECON® 200 rather than three. (NOTE: the alternate procedures were not used if high efficacy of decontamination was observed even with a less-than-target application rate with the primary procedures.)

Alternative Procedures:

- Apply the EasyDECON® 200 solution to the test coupons (or SFW to the positive control coupons) from a distance of 30.5 cm using the handheld spray bottle, until all test coupon surfaces are fully wetted by the solution.

For nonporous materials (steel, aluminum, glass, porcelain, granite):

- Reapply the EasyDECON® 200 5 minutes after the first application, and again 10, 15, 20, and 25 minutes after the first application. Perform additional applications as needed if the test coupon surfaces become dry.
- When 30 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any EasyDECON® 200 solution pooled on the test coupon.

For porous materials (concrete, brick, asphalt paving, treated wood, butyl rubber):

- Reapply the EasyDECON® 200 10 minutes after the first application, and again 20, 30, 40, and 50 minutes after the first application. Perform additional applications as needed if the test coupon surfaces become dry.
- When 60 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any EasyDECON® 200 solution pooled on the test coupon.

Appendix E
Preparation and Application of Spor-Klenz® RTU

General Description

Spor-Klenz® Ready to Use (RTU) is a liquid decontaminant consisting of 1.00 % hydrogen peroxide (H_2O_2), 0.08% peroxyacetic acid, and < 10% acetic acid in aqueous solution. Spor-Klenz® RTU is designed to be used directly, without dilution. The product is a clear, colorless liquid with a pH of 1.5 to 2.0 and a density of approximately 1.0 g/mL.

Sodium thiosulfate (STS) was used to stop the action of Spor-Klenz® RTU so that efficacy could be determined. Trial runs were conducted before efficacy testing to establish the appropriate concentration of STS for neutralization of Spor-Klenz® RTU.

Application Procedure for Testing

An application procedure for use of Spor-Klenz® RTU in testing was developed based on information provided on the product label, in particular for its use as a sporicide. The aim is to use a relatively simple application process that is likely to be effective when carried out with conventional and readily available equipment.

Spor-Klenz® RTU was applied to test coupons using a hand-held plastic spray bottle. A similar bottle was used to apply SFW to positive control test coupons. The step-by-step application procedure was as indicated below. Note that the procedure for porous materials differed from that for nonporous materials.

- Apply Spor-Klenz® RTU to the test coupons (or SFW to the positive control coupons) from a distance of 30.5 cm using the handheld spray bottle, until all test coupon surfaces are fully wetted by the solution.

For nonporous materials (steel, aluminum, glass, porcelain, granite):

- Reapply Spor-Klenz® RTU as needed to keep the test coupon surfaces wetted throughout the test.
- When 30 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any Spor-Klenz® RTU pooled on the test coupon.

For porous materials (concrete, brick, asphalt paving, treated wood, butyl rubber):

- Reapply the Spor-Klenz® RTU as needed to keep the test coupon surfaces wetted throughout the test.
- Regardless of the wetness of the coupons, reapply SporKlenz® 30 minutes after the first application.
- When 60 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any Spor-Klenz® RTU pooled on the test coupon.

Appendix F
Preparation and Application of Peridox® RTU

General Description

Peridox® RTU is a liquid decontaminant consisting of 4.0 to 4.5 % hydrogen peroxide (H_2O_2) and 0.17 to 0.22% peracetic acid, in aqueous solution. Peridox® RTU is intended to be used as is, without further dilution. The product is a colorless liquid with a pH of about 2.2 and a density of approximately 1.02 g/mL.

Sodium thiosulfate (STS) was used to stop the action of Peridox® RTU so that efficacy could be determined. Trial runs were conducted before efficacy testing to establish the appropriate concentration of STS for neutralization of Peridox® RTU.

Application Procedure for Testing

An application procedure for use of Peridox® RTU in testing was developed based on information provided by the vendor. The aim is to use a relatively simple application process that is likely to be effective when carried out with conventional and readily available equipment.

Peridox® RTU was applied to test coupons using a hand-held plastic spray bottle. A similar bottle was used to apply SFW to positive control test coupons. The step-by-step application procedure was as indicated below. Note that the procedure for porous materials differed from that for non-porous materials.

- Apply Peridox® RTU to the test coupons (or SFW to the positive control coupons) from a distance of 30.5 cm using the handheld spray bottle, until all test coupon surfaces are fully wetted by the solution.

For nonporous materials (steel, glass, aluminum, porcelain, granite):

- Reapply Peridox® RTU as needed to keep the test coupon surfaces wetted.
- When 30 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any Peridox® RTU pooled on the test coupon.

For porous materials (concrete, brick, asphalt paving, treated wood, butyl rubber):

- Reapply the Peridox® RTU as needed to keep the test coupon surfaces fully wetted.
- When 60 minutes have elapsed since the first application, place each coupon into the extraction solution (containing the pre-determined amount of STS neutralization solution) along with any Peridox® RTU pooled on the test coupon)

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